DEVELOPMENT AND STANDARDIZATION OF POLYHERBAL ANTIDIABETIC FORMULATION

A dissertation submitted to

THE TAMILNADU Dr. M.G.R MEDICAL UNIVERSITY

CHENNAI-600 032

In partial fulfillment of the requirements for the award of degree of

MASTER OF PHARMACY

IN

BRANCH III - PHARMACOGNOSY

Submitted by

P.VIJAYALAKSHMI

REG. NO: 261620659

Under the guidance of

DR. P. MUTHUSAMY, M.Pharm., Ph.D., B.L.,

Department of Pharmacognosy

College of Pharmacy

Madras Medical College

Chennai-600 003

MAY 2018

COLLEGE OF PHARMACY
MADRAS MEDICAL COLLEGE
CHENNAI – 600 003
TAMIL NADU

CERTIFICATE

This is to certify that the dissertation entitled **"DEVELOPMENT AND STANDARDIZATION OF POLYHERBAL ANTIDIABETIC FORMULATION"** submitted by **P.VIJAYALAKSHMI, Reg. No: 261620659** to the Tamil Nadu Dr. M.G.R Medical University examinations is evaluated.

EXAMINERS

1.

2.

Place: Chennai-03

Date:

COLLEGE OF PHARMACY
MADRAS MEDICAL COLLEGE
CHENNAI – 600 003
TAMIL NADU

DR. A. JERAD SURESH, M.Pharm., Ph.D., MBA.,

Principal,

College of Pharmacy,

Madras Medical College,

Chennai-600003.

CERTIFICATE

This is to certify that the dissertation entitled **"DEVELOPMENT AND STANDARDIZATION OF POLYHERBAL ANTIDIABETIC FORMULATION"** submitted by **P.VIJAYALAKSHMI, Reg. No: 261620659** in partial fulfillment of the requirements for the award of the degree of **MASTER OF PHARMACY IN PHARMACOGNOSY** by The Tamil Nadu Dr. M.G.R Medical University, Chennai is a bonafide record of work done by her in the Department of Pharmacognosy, College of Pharmacy, Madras Medical College, Chennai-600003 during the academic year **2017-2018** under the guidance of **DR. P. Muthusamy, M. Pharm., Ph.D., BL.,** Assistant Professor Department of Pharmacognosy, College of Pharmacy, Madras Medical College, Chennai-600 003.

DR. A. JERAD SURESH, M.Pharm., Ph.D., MBA.

Place: Chennai-03

Date:

COLLEGE OF PHARMACY
MADRAS MEDICAL COLLEGE
CHENNAI – 600 003
TAMIL NADU

DR. R. RADHA, M.Pharm., Ph.D., M.B.A.,

Professor and Head,

Department of Pharmacognosy,

College of Pharmacy,

Madras Medical College,

Chennai- 600 003.

CERTIFICATE

This is to certify that the dissertation entitled **"DEVELOPMENT AND STANDARDIZATION OF POLYHERBAL ANTIDIABETIC FORMULATION"** submitted by **P. VIJAYALAKSHMI**, **Reg. No: 261620659** in partial fulfillment of the requirements for the award of the degree of **MASTER OF PHARMACY IN PHARMACOGNOSY** by The Tamil Nadu Dr. M.G.R Medical University, Chennai, is a bonafide record of work done by her in the Department of Pharmacognosy, College of Pharmacy, Madras Medical College, Chennai-600 003 during the academic year **2017-2018** under the guidance of **DR. P. Muthusamy, M.Pharm., Ph.D., B.L.,** Assistant Professor, Department of Pharmacognosy, College of Pharmacy, Madras Medical College, Chennai-600 003.

DR. R. RADHA, M.Pharm., Ph.D., M.B.A.,

Place: Chennai-03

Date:

COLLEGE OF PHARMACY
MADRAS MEDICAL COLLEGE
CHENNAI – 600 003
TAMIL NADU

DR. P. MUTHUSAMY, M. Pharm., Ph.D., B.L.,

Assistant Professor,

Department of Pharmacognosy,

College of Pharmacy,

Madras Medical College,

Chennai -600003.

CERTIFICATE

This is to certify that the dissertation entitled **"DEVELOPMENT AND STANDARDIZATION OF POLYHERBAL ANTIDIABETIC FORMULATION"** submitted by **P.VIJAYALAKSHMI**, **Reg. No: 261620659** in partial fulfillment of the requirements for the award of the degree of **MASTER OF PHARMACY IN PHARMACOGNOSY** by The Tamil Nadu Dr. M.G.R Medical University, Chennai, is a bonafide record of work done by her in the Department of Pharmacognosy, College of Pharmacy, Madras Medical College, Chennai-600003, during the academic year **2017 - 2018** under my guidance and supervision.

DR. P. MUTHUSAMY, M.Pharm, Ph.D., B.L.,

Place: Chennai-03

Date:

ACKNOWLEDGEMENT

I extend my sincere thanks to **God** and I Wish to acknowledge my sincere thanks and express my heartful gratitude to the following persons with whose help and encouragement, I have completed this project work successfully.

I express my sincere gratitude and thanks to our beloved **Dean DR. R. Jayanthi, MD. F.R.C.P.,(Glasg),** Madras Medical College, Chennai - 03 for her kind wishes and blessings providing the necessary facilities to carry out my project work.

I acknowledge my sincere thanks to **Prof. Dr.A.Jerad Suresh, M.Pharm., Ph.D.,** Principal, College of Pharmacy, Madras Medical college for his continuous support in carrying out my dissertation work in this institution.

I consider myself very much lucky with profound privilege and great pleasure in expressing our deep sense of gratitude to **Prof. Dr. R. Radha, M.Pharm. Ph.D, M.B.A.,** Professor and Head, Department of Pharmacognosy, College of Pharmacy, Madras Medical College, Chennai for her supportive suggestion and encouragement to perform better.

It's my privilege to acknowledge with deep sense of gratitude and sincere thanks to my guide **Dr. P. Muthusamy, M.Pharm, Ph.D., BL.,** Assistant Professor, Department of Pharmacognosy, College of Pharmacy, Madras Medical College, Chennai for his interest, valuable suggestions, wise criticism and constant inspiration throughout the progress of my work.

It's a great pleasure for me to acknowledge my sincere thanks to all my teachers, **Dr. R. Vijayabharathi, M.Pharm, Ph.D., Dr. R. Vadivu, M.Pharm, Ph.D.,** and **Mrs. B. Kumuthaveni, M.Pharm.,** of the Department of Pharmacognosy for their valuable support and excellent co-operation when needed.

I acknowledge my sincere thanks to **Dr. S. Rajaraman, M.V.Sc., Ph.D., Special Veterinary Officer, Animal Expiremental laboratory,** Madras Medical College, Chennai - 3 and **Mr. Kandasamy,** Assistant of Animal Experimental House, Madras Medical College, for their continuous support in carrying out my dissertation work in this institution.

My special thanks to **Dr. R. Suresh, M. Pharm, Ph.D., Dr. R. Sowmya, M.sc., M.Phil, Ph.D.,** and **Mr. P. Parthasarathy, M,Sc., Greensmed Lab,** Thoraipakkam, Chennai-97 for their valuable support, guidance and providing necessary facilities, chemicals and help during my entire project work.

I express my sincere thanks to **Government of Tamilnadu** for providing to giving this oppourtunity.

I take this opportunity to my thanks to **Dr. K.N. Sunilkumar, Research Officer** and **HOD, Pharmacognosy, Central Siddha Research Institute, Arumbakkam, Chennai - 106** for identification and evaluation of this plant material.

I thank Mrs. **T.S. Lakshmi and Mrs. M. Kumudha** Lab Technicians, Department of Pharmacognosy, Madras Medical College, Chennai for providing me the necessary equipment and reagents to fullfill my researchwork.

I extend my sincere thanks to my senior **S. Shanmugapriya** for her guidance and clarifying my every doubts now and then during project.

I express my hearty gratitude to my freinds of **Pharmaceutics Department** for providing timely help during formulation process. I express my hearty gratitude to my Bacthmates **Mr. K.Anandhan, S. Jeya shanmuga priya, B. Prasannadevi, K. Rajakumar, M. Srinivasan, M. Senthamil Kavitha, M. Thangamani R. Vasanthi and my first year M. Pharm freinds** for encouraging me continuously to complete my project.

Last but not least, I would like to dedicate this work to the people in my life that I appreciate and love more than words can say: My sons **(Rithish& Vishva**), My father (**Panneer**), My husband (**Rajakumar**), My mother (**Gowri**), My mother in law(**Kuppammal**), My brother(**Sivakumar**) and his wife (**Priyadharshni**),My Sister (**Sathya**) and her family members and my friends for their unconditional love, sacrifices, encouragements, supports and “patience”.

P. Vijayalakshmi,
Reg. No. 261620659

CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. INTRODUCTION

1.1 HERBAL MEDICINE[1,2]

Herbal medicine have become the remedy for most of the diseases. In conjunction with a healthy diet and lifestyle they target specific health goals providing every cell the most appropriate and optimal nourishment. These herbal supplements do not have any harmful side effects that might disturb physical health unlike synthetics. For every synthetic drug present there is an alternative herbal drug. Man in his everlasting search for cure of serious illnesses, at last finds his way to our indigenous medicine.

Indigenous system of medicine which is also known as traditional or folk medicine encompasses of medical knowledge systems that have germinated over generations within various societies before the era of modern medicine. Indigenous medicines include Herbal, Ayurveda, Siddha medicine, Unani, ancient Iranian medicine, Islamic Medicine, Traditional Chinese medicine, Traditional Vietnamese medicine, Acupuncture, Muti, Ifa, Traditional African medicine, and other medicinal practices all over the world.

The World Health Organization (WHO) defines traditional medicine as:

"The health practices, approaches, knowledge and beliefs incorporating plant, animal and mineral-based medicines, spiritual therapies, manual techniques and exercises, applied singularly or in combination to treat, diagnose and prevent illnesses or maintain well-being."

From available literature, the use of herbs dates back 5,000 years to the ancient Sumerians, who described well-established medicinal uses for plants. Nature always stands as a golden mark to exemplify the outstanding phenomena of symbiosis. Nowadays people are well versed with the potency and side effects of synthetic drugs. Hence, there is an increasing interest in the natural product remedies with a basic approach towards the nature. Natural products obtained from plant,

animal and minerals have been the backbone of the treatment of human diseases. At about 80 % of people in developing countries still hinge upon traditional medicine, based largely on species of plants and animals for their primary health care. Indigenous system of medicine is the need of the day.

1.2 IMPORTANCE[3-5]

Ayurveda is a traditional system of medicine using a wide range of modalities to create health and wellbeing. The main aspire of Ayurveda health care is to restore the physical mental and emotional balance in patients, thus improving the health, preventing disease and to treat any current illness. The number of patients looking for alternate and herbal therapy is growing exponentially. Thus the herbal medicines are now in great demand in the developing world for primary healthcare not only for its inexpensiveness but also for better cultural acceptability; better compatibility with the human body and minimal side effects.

Herbal medicine is still the mainstay of about 75 – 80% of the world population for primary healthcare mainly in the developing countries. However among the estimated 250,000 – 400,000 of plant species, only about 6% have been studied for biological activity, and about 15% have been investigated based on its phytochemicals. Therefore it seems necessary to evaluate the herbs properly.

The first reason for the use of herbals is that it is part of the culture and belief of some people for maintenance of health or to treat certain ailments. The second reason for the increased use of herbals is the relatively cheaper cost of herbal products and hence affordability to the lower income group. The third reason is that the public has the impression of herbals being natural and that anything natural is safe. There is also this notion that herbal products do not contain chemicals and only those chemicals found in modern medicines, are linked to toxicity, and hence are more harmful.

1.3 FORMULATION OF HERBAL PRODUCTS[6]

An herbal "formula" consists of a selective combination of individual herbal ingredients that are formulated for a specific ailment or group of disease-conditions. When herbs are combined together, they become more potent and effective within the body than single herb due to their activating or catalyzing influence upon one another. These combinations acts as powerful catalysts in order to activate over own individual healing energies (or vital force) which permeate the entire organism and reside in each and every cell in our bodies.

1.3.1 Advantages of Herbal Formulations:

There are a number of advantages associated with using herbal medicines as opposed to allopathic products. Examples include the following:

• **Reduced risk of side effects:** Most herbal medicines are well tolerated by the patient, with fewer unintended consequences than pharmaceutical drugs. Herbs typically have fewer side effects than traditional medicine, and may be safer to use over time.

WHO Guidelines for Standardization of Herbal Formulation[7,8]

Standardization is an important aspect for maintaining and assessing the quality and safety of the polyherbal formulation as these are combinations of more than one herb to attain the desire therapeutic effect. Standardization minimizes batch to batch variation, assures safety, efficacy, quality and acceptability of the polyherbal formulations. Standardisation involves:

- Quality control of crude drugs material, plant preparations and finished products.
- Stability assessment and shelf life.
- Safety assessment, documentation of safety based on experience or toxicological studies.
- Assessment of efficacy by ethnomedical informations and biological activity evaluations

1.3.2. Capsule formulated with herbs [6,9]

Figure :1 Capsule formulated with herbs

Herbal products can be formulated in the form of Capsules, Tablets, Syrups, Lehiyas, Tailas, Bhasmas, Powders etc. The most versatile form is the capsule form that provides conventional drug delivery for controlling absorption, enhancing bioavailability and dosage accuracy. It is easier to consume a formulated capsule containing active ingredients than to swallow the herbs as such.

The word “capsule” in the English language is derived from the Latin word “Capsula”, which means a small box or container in more recent times, capsule has been used primarily to describe a solid oral dosage form, which consists of a container,usually made of gelatin filled with a medicinal substance. There are many forms of capsules and they can be divided into two main categories, which in current English usage are described by the adjectives “hard” and “soft”. The “hard capsule” consists of two separate parts, each semi-closed cylinder in shape. One part the “cap” has a slightly larger diameter than the other, which is called the “body” and is longer the cap fits closely over the body to form a sealed unit.

1.4. Diabetes mellitus[10-13]

As per WHO, Diabetes Mellitus is defined as heterogenous metabolic disorder characterised by common feature of chronic hyperglycemia with disturbance of carbohydrate, protein and fat metabolism.

Types of diabetes

- **Type - I diabetes** (insulin dependent diabetes mellitus)
- **Type - II diabetes** (formerly, non-insulin dependent diabetes mellitus)
- **Gestational diabetes** (first recognition during pregnancy)
- **Diabetes due to other causes** (genetic defects or medication)

Type 1 diabetes (formerly known as insulin-dependent) in which the pancreas fails to produce the insulin which is essential for survival. This form develops most frequently in children and adolescents, but is being increasingly noted later in life.

Type 2 diabetes (formerly named non-insulin-dependent) which results from the body's inability to respond properly to the action of insulin produced by the pancreas. Type 2 diabetes is much more common and accounts for around 90% of all diabetes cases worldwide. It occurs most frequently in adults, but is being noted increasingly in adolescents as well.

Certain genetic markers have been shown to increase the risk of developing Type 1 diabetes. Type 2 diabetes is strongly familial, but it is only recently that some genes have been consistently associated with increased risk for Type 2 diabetes in certain populations. Both types of diabetes are complex diseases caused by mutations in more than one gene, as well as by environmental factors.

GESTATIONAL DIABETES

Diabetes that's triggered by pregnancy is called gestational diabetes (pregnancy, to some degree, leads to insulin resistance). It is often diagnosed in

middle or late pregnancy. Because high blood sugar levels in a mother are circulated through the placenta to the baby, gestational diabetes must be controlled to protect the baby's growth and development.

The rate of gestational diabetes is between 2% to 10% of pregnancies. Gestational diabetes usually resolves itself after pregnancy. Up to 10% of women with gestational diabetes develop type 2 diabetes. It can occur anywhere from a few weeks after delivery to months or years later.

Diabetes in pregnancy[gestational diabetes] may give rise to several adverse outcomes, including congenital malformations, increased birth weight and an elevated risk of perinatal mortality. Strict metabolic control may reduce these risks to the level of those of non-diabetic expectant mothers.

Symptoms

The symptoms of diabetes may be pronounced, subdued, or even absent.

- In Type 1 diabetes, the classic symptoms are excessive secretion of urine (polyuria), thirst (polydipsia), weight loss and tiredness.
- These symptoms may be less marked in Type 2 diabetes. In this form, it can also happen that no early symptoms appear and the disease is only diagnosed several years after its onset, when complications are already present.

Epidemiology of diabetes

The incidence of diabetic is growing rapidly in United States and worldwide. Globally as of 2010, an estimated 285 million people had diabetes, with type II making up about 90% of the cases. In 2013, according to International Diabetes Federation an estimated 381 million people had diabetes, its prevalence is increasing rapidly. It is estimated that more than 250 million people worldwide are afflicted with diabetes and the prevalence is expected to exceed 350 million by the year 2030.

PATHOPHYSIOLOGY

Pancreas

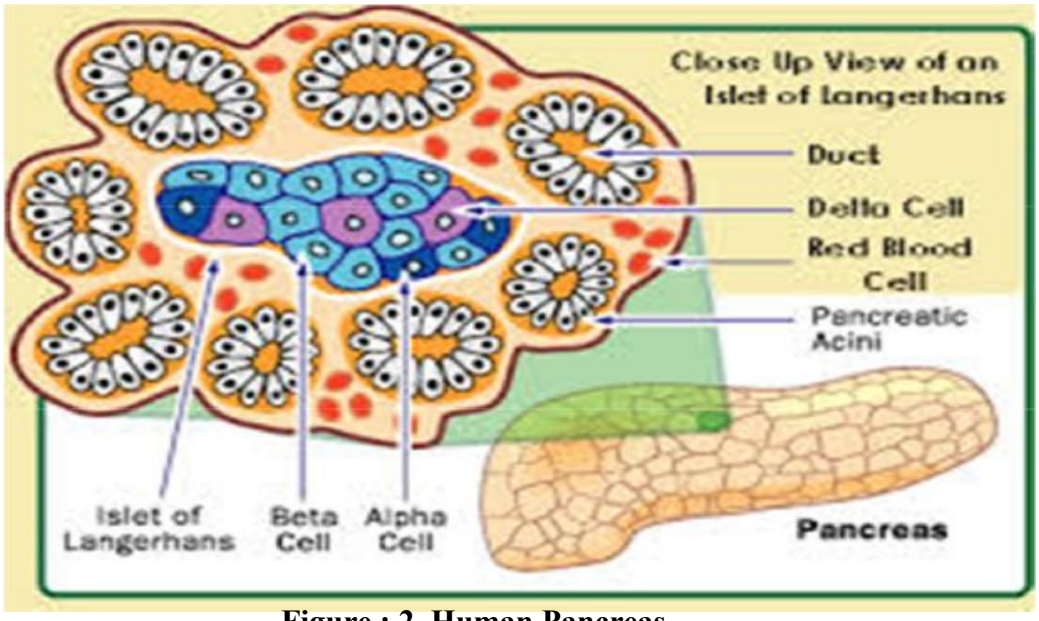


Figure : 2 Human Pancreas

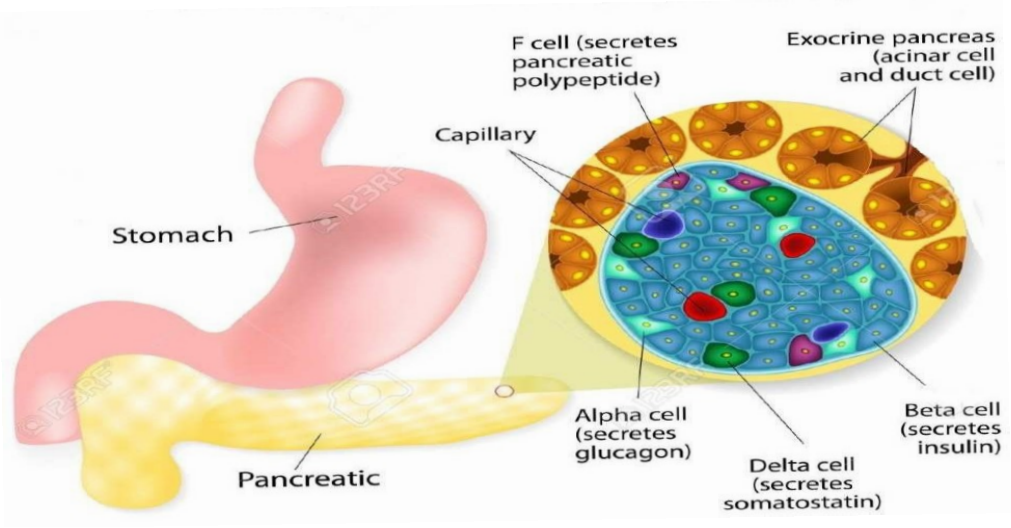


Figure: 3 Langerhans

The hormones play an important role in regulating the metabolic activities of the body, particularly the hameostasis of blood glucose. The pancreas is both an endocrine and exocrine gland, in which endocrine produces the peptide hormone insulin, glucagon and somatostatin and exocrine gland produces digestive enzymes The peptide hormones are secreted from cells located in the islet of langerhans (β cells produce insulin, alpha cells produces glucagon and δ cells produce somatostatin).

Insulin

Insulin was discovered in 1921 by Banting and best who demonstrated the hypoglycaemic action of an extract of pancreas. In 1922 an extract containing insulin was first used on a 14 year old boy suffering from severe diabetes mellitus with excellent response. Insulin was then purified in a few years.

Insulin Structure

Insulin is composed of two chains of amino acids named chain A (21 amino acids) and chain B (30 amino acids) that are linked together by two disulfide bridges. There is a 3rd disulfide bridge within the A chain that links the 6th and 11th residues of the A chain together.

In most species, the length and amino acid compositions of chains A and B are similar, and the positions of the three disulfide bonds are highly conserved. For this reason, pig insulin can be used to replace deficient human insulin levels in diabetes patients. Today, porcine insulin has largely been replaced by the mass production of human proinsulin by bacteria (recombinant insulin).

Insulin secretion

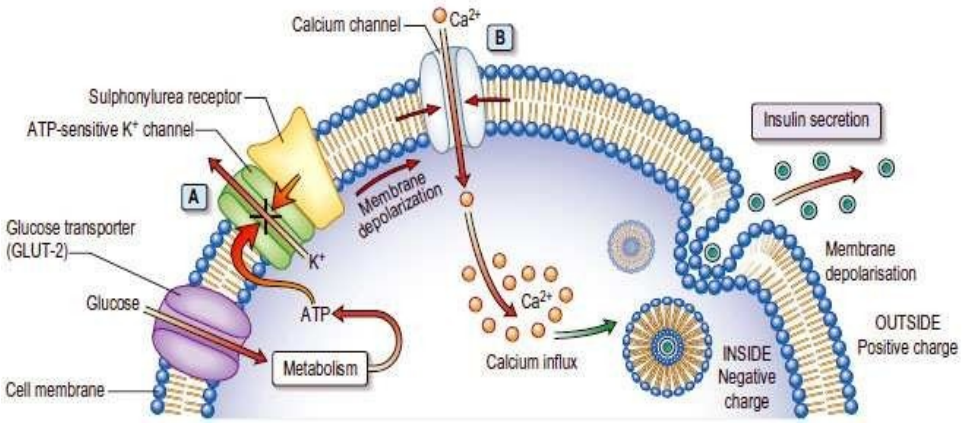
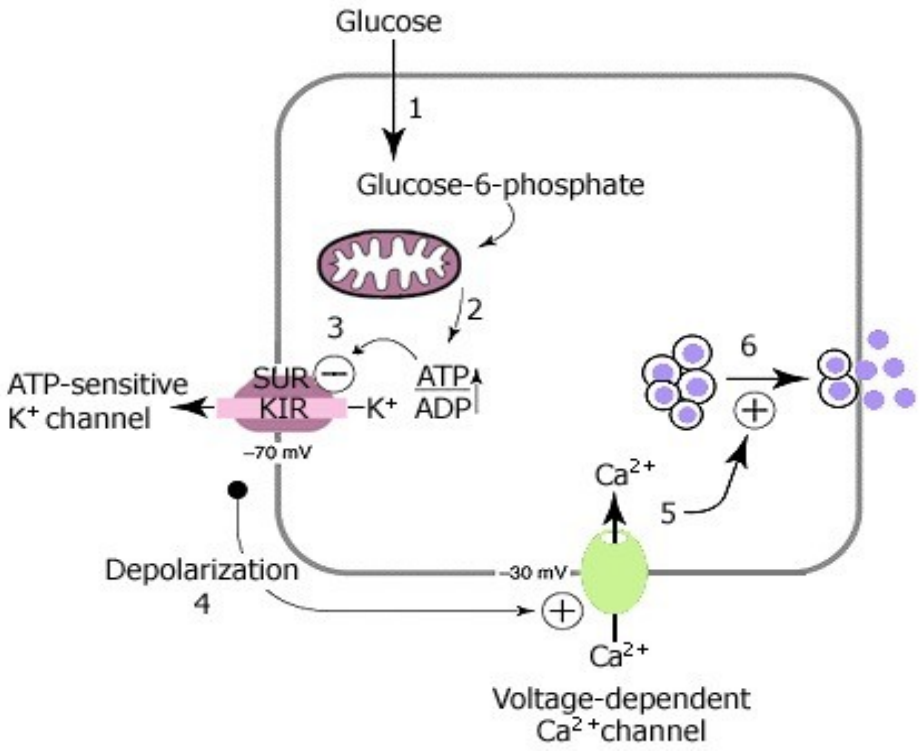


Figure:4 Secretion of Insulin in β cells

The insulin-making cells of the body are called beta cells, and they are found in the pancreas gland. These cells clump together to form the "islets of Langerhans", named for the German medical student who described them.

Rising levels of glucose inside the pancreatic beta cells trigger the release of insulin:



1. Glucose is transported into the beta cell by type 2 glucose transporters (GLUT2). Once inside, the first step in glucose metabolism is the phosphorylation of glucose to produce glucose-6-phosphate. This step is catalyzed by glucokinase—it is the rate-limiting step in glycolysis, and it effectively traps glucose inside the cell.
2. As glucose metabolism proceeds, ATP is produced in the mitochondria.
3. The increase in the ATP:ADP ratio closes ATP-gated potassium channels in the beta cell membrane. Positively charged potassium ions (K^+) are now prevented from leaving the beta cell.
4. The rise in positive charge inside the beta cell causes depolarization.
5. Voltage-gated calcium channels open, allowing calcium ions (Ca^{2+}) to flood into the cell.
6. The increase in intracellular calcium concentration triggers the secretion of insulin via exocytosis.

There are two phases of insulin release in response to a rise in glucose. The first is an immediate release of insulin. This is attributable to the release of preformed insulin, which is stored in secretory granules. After a short delay, there is a second, more prolonged release of newly synthesized insulin.

Once released, insulin is active for a only a brief time before it is degraded by enzymes. Insulinase found in the liver and kidneys breaks down insulin circulating in the plasma, and as a result, insulin has a half-life of only about 6 minutes. This short duration of action allows rapid changes in the circulating levels of insulin.

Insulin Receptor

The net effect of insulin binding is to trigger a cascade of phosphorylation and dephosphorylation reactions. These actions are terminated by dephosphorylation of the insulin receptor.

Similar to the receptors for other polypeptide hormones, the receptor for insulin is embedded in the plasma membrane and is composed of a pair of alpha subunits and a pair of beta subunits. The alpha subunits are extracellular and contain the insulin-binding site. The beta subunits span the membrane and contain the enzyme tyrosine kinase. Kinases are a group of enzymes that phosphorylate proteins (the reverse reaction is catalyzed by a group of enzymes called phosphatases).

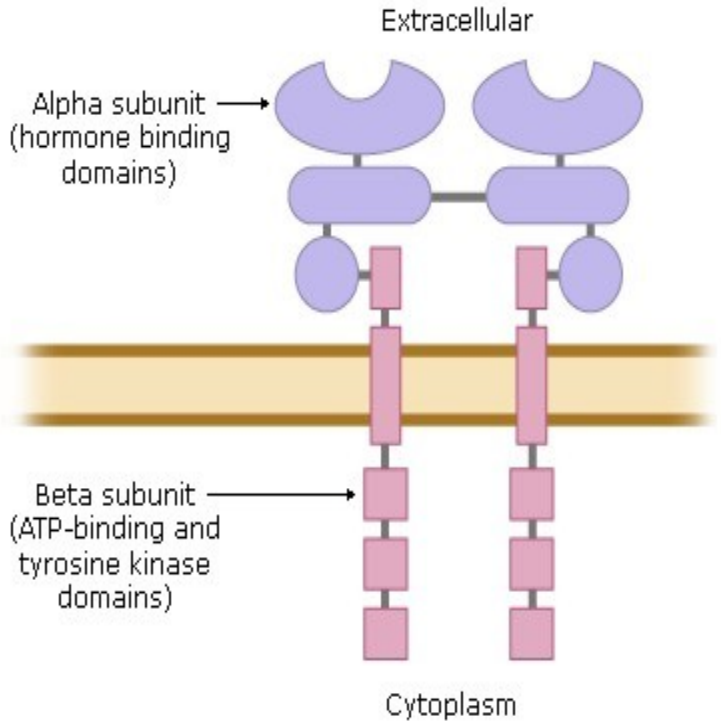


Figure: 5 The insulin receptor

The insulin receptor is a tyrosine kinase receptor and is composed of a pair of alpha subunits and a pair of beta subunits. Insulin binds to the alpha subunits and induces a conformational change that is transmitted to the beta subunits that autophosphorylate and initiate a cascade of phosphorylation and dephosphorylation reactions.

Insulin binding to the alpha subunits induces a conformational change that is transmitted to the beta subunits and causes them to phosphorylate themselves (autophosphorylation). A specific tyrosine of each beta subunit is phosphorylated along with other target proteins, such as insulin receptor substrate (IRS). As these and other proteins inside the cell are phosphorylated, this in turn alters their activity, bringing about the wide biological effects of insulin.

Insulin Action

The binding of insulin results in a wide range of actions that take place over different periods of time. Almost immediately, insulin promotes the uptake of glucose into many tissues that express GLUT4 glucose transporters, such as skeletal muscle and fat. Insulin increases the the activity of these transporters and increases their numbers by stimulating their recruitment from an intracellular pool to the cell surface. Not all tissues require insulin for glucose uptake. Tissues such as liver cells, red blood cells, the gut mucosa, the kidneys, and cells of the nervous system use a glucose transporter that is not insulin dependent.

Over minutes to hours, insulin alters the activity of various enzymes as a result of changes in their phosphorylation status.

Over a period of days, insulin increases the amounts of many metabolic enzymes. These reflect an increase in gene transcription, mRNA, and enzyme synthesis.

After a Meal—the Role of Insulin

The rise in blood glucose following a meal is detected by the pancreatic beta cells, which respond by releasing insulin. Insulin increases the uptake and use of glucose by tissues such as skeletal muscle and fat cells. This rise in glucose also inhibits the release of glucagon, inhibiting the production of glucose from other sources, e.g., glycogen break down.

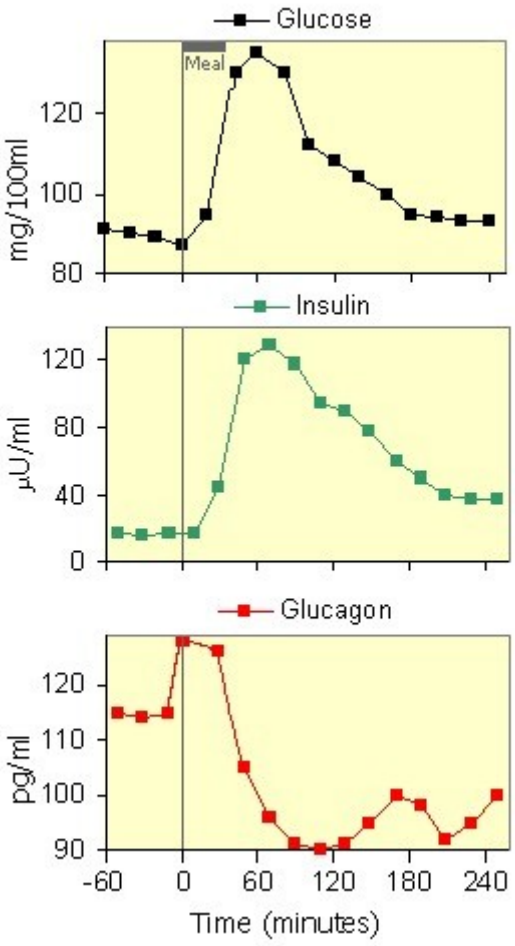


Figure: 6 Changes in key hormones after a meal

Changes in blood levels of glucose, insulin, and glucagon after a carbohyrate-rich meal (ingested at time 0 minutes

1. Use Glucose

Once inside the cell, some of the glucose is used immediately via glycolysis. This is a central pathway of carbohydrate metabolism because it occurs in all cells in the body, and because all sugars can be converted into glucose and enter this pathway. During the well-fed state, the high levels of insulin and low levels of glucagon

stimulate glycolysis, which releases energy and produces carbohydrate intermediates that can be used in other metabolic pathways.

2. Make Glycogen

Any glucose that is not used immediately is taken up by the liver and muscle where it can be converted into glycogen (glycogenesis). Insulin stimulates glycogenesis in the liver by:

- stimulating hepatic glycogen synthetase (the enzyme that catalyzes glycogen synthesis in the liver)
- inhibiting hepatic glycogen phosphorylase (the enzyme that catalyzes glycogen breakdown in the liver)
- inhibiting glucose synthesis from other sources (inhibits gluconeogenesis)

Insulin also encourages glycogen formation in muscle, but by a different method. Here it increases the number of glucose transporters (GLUT4) on the cell surface. This leads to a rapid uptake of glucose that is converted into muscle glycogen.

3. Make Fat

When glycogen stores are fully replenished, excess glucose is converted into fat in a process called lipogenesis. Glucose is converted into fatty acids that are stored as triglycerides (three fatty acid molecules attached to one glycerol molecule) for storage. Insulin promotes lipogenesis by:

- increasing the number of glucose transporters (GLUT4) expressed on the surface of the fat cell, causing a rapid uptake of glucose
- increasing lipoprotein lipase activity, which frees up more fatty acids for triglyceride synthesis

In addition to promoting fat synthesis, insulin also inhibits fat breakdown by inhibiting hormone-sensitive lipase (an enzyme that breaks down fat stores). As a result, there are lower levels of fatty acids in the blood stream.

Insulin also has an anabolic effect on protein metabolism. It stimulates the entry of amino acids into cells and stimulates protein production from amino acids.

Fasting—the Role of Glucagon

Fasting is defined as more than eight hours without food. The resulting fall in blood sugar levels inhibits insulin secretion and stimulates glucagon release. Glucagon opposes many actions of insulin. Most importantly, glucagon raises blood sugar levels by stimulating the mobilization of glycogen stores in the liver, providing a rapid burst of glucose. In 10–18 hours, the glycogen stores are depleted, and if fasting continues, glucagon continues to stimulate glucose production by favouring the hepatic uptake of amino acids, the carbon skeletons of which are used to make glucose.

In addition to low blood glucose levels, many other stimuli stimulate glucagon release including eating a protein-rich meal (the presence of amino acids in the stomach stimulates the release of both insulin and glucagon, glucagon prevents hypoglycemia that could result from unopposed insulin) and stress (the body anticipates an increased glucose demand in times of stress).

"Starvation in the Midst of Plenty"

Diabetes is often referred to as "starvation in the midst of plenty" because the intracellular levels of glucose are low, although the extracellular levels may be extremely high.

As in starvation, type 1 diabetics use non-glucose sources of energy, such as fatty acids and ketone bodies, in their peripheral tissues. But in contrast to the starvation state, the production of ketone bodies can spiral out of control. Because the

ketones are weak acids, they acidify the blood. The result is the metabolic state of diabetic ketoacidosis (DKA). Hyperglycemia and ketoacidosis are the hallmark of type 1 diabetes.

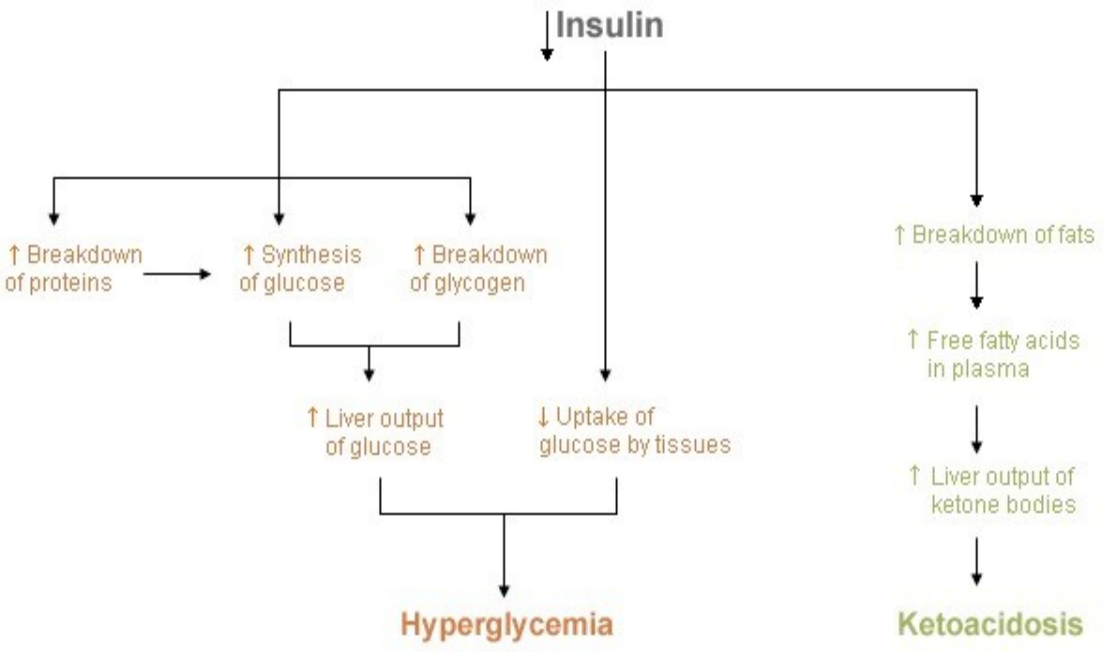


Figure: 7 Metabolic changes in Ketoacidosis

Hyperglycemia is caused by the increased production of glucose by the liver (driven by glucagon) and the decreased use of glucose of insulin by peripheral tissues (because of the lack of insulin

Hypertriglyceridemia is also seen in DKA. The liver combines triglycerol with protein to form very low density lipoprotein (VLDL). It then releases VLDL into the blood. In diabetics, the enzyme that normally degrades lipoproteins (lipoprotein lipase) is inhibited by the low level of insulin and the high level of glucagon. As a result, the levels of VLDL and chylomicrons (made from lipid from the diet) are high in DKA.

Prevalence

- Recently compiled data show that approximately 150 million people have diabetes mellitus worldwide, and that this number may well double by the year 2025. Much of this increase will occur in developing countries and will be due to population growth, ageing, unhealthy diets, obesity and sedentary lifestyles.
- By 2025, while most people with diabetes in developed countries will be aged 65 years or more, in developing countries most will be in the 45-64 year age bracket and affected in their most productive years.

Diagnosis

- WHO has published recommendations on diagnostic values for blood glucose concentration. The diagnostic level of fasting blood glucose concentration was last modified in 1999.

Treatment

- The mainstay of non-pharmacological diabetes treatment is diet and physical activity.
- About 40% of diabetes sufferers require oral agents for satisfactory blood glucose control, and some 40% need insulin injections. This hormone was isolated by Frederic Banting and Charles Best in 1921 in Canada. It revolutionized the treatment of diabetes and prevention of its complications, transforming Type 1 diabetes from a fatal disease to one in which long-term survival became achievable.
- People with Type 1 diabetes are usually totally dependent on insulin injections for survival. Such people require daily administration of insulin. The majority of people suffering from diabetes have the Type 2 form. Although they do not depend on insulin for survival, about one third of sufferers needs insulin for reducing their blood glucose levels.

Complications associated with diabetes mellitus

- Diabetic retinopathy is a leading cause of blindness and visual disability. Diabetes mellitus is associated with damage to the small blood vessels in the retina, resulting in loss of vision. Findings, consistent from study to study, make it possible to suggest that, after 15 years of diabetes, approximately 2% of people become blind, while about 10% develop severe visual handicap. Loss of vision due to certain types of glaucoma and cataract may also be more common in people with diabetes than in those without the disease.
- Good metabolic control can delay the onset and progression of diabetic retinopathy. Loss of vision and blindness in persons with diabetes can be prevented by early detection and treatment of vision-threatening retinopathy: regular eye examinations and timely intervention with laser treatment, or through surgery in cases of advanced retinopathy. There is evidence that, even in developed countries, a large proportion of those in need is not receiving such care due to lack of public and professional awareness, as well as an absence of treatment facilities. In developing countries, in many of which diabetes is now common, such care is inaccessible to the majority of the population.
- Diabetes is among the leading causes of kidney failure, but its frequency varies between populations and is also related to the severity and duration of the disease. Several measures to slow down the progress of renal damage have been identified. They include control of high blood glucose, control of high blood pressure, intervention with medication in the early stage of kidney damage, and restriction of dietary protein. Screening and early detection of diabetic kidney diseae are an important means of prevention.
- Heart disease accounts for approximately 50% of all deaths among people with diabetes in industrialized countries. Risk factors for heart disease in people with diabetes include smoking, high blood pressure, high serum cholesterol and obesity. Diabetes negates the protection from heart disease

which pre-menopausal women without diabetes experience. Recognition and management of these conditions may delay or prevent heart disease in people with diabetes.

- Diabetic neuropathy is probably the most common complication of diabetes. Studies suggest that up to 50% of people with diabetes are affected to some degree. Major risk factors of this condition are the level and duration of elevated blood glucose. Neuropathy can lead to sensory loss and damage to the limbs. It is also a major cause of impotence in diabetic men.
- Diabetic foot disease, due to changes in blood vessels and nerves, often leads to ulceration and subsequent limb amputation. It is one of the most costly complications of diabetes, especially in communities with inadequate footwear. It results from both vascular and neurological disease processes. Diabetes is the most common cause of non-traumatic amputation of the lower limb, which may be prevented by regular inspection and good care of the foot.

Prevention

Large, population-based studies in China, Finland and USA have recently demonstrated the feasibility of preventing, or delaying, the onset of diabetes in overweight subjects with mild glucose intolerance (IGT). The studies suggest that even moderate reduction in weight and only half an hour of walking each day reduced the incidence of diabetes by more than one half.

Diabetes is a serious and costly disease which is becoming increasingly common, especially in developing countries and disadvantaged minorities. However, there are ways of preventing it and/or controlling its progress. Public and professional awareness of the risk factors for, and symptoms of diabetes are an important step towards its prevention and control.

1.5. HERBAL DRUGS FOR DIABETES MELLITUS[14-16]

In the Ayurvedic system of medicine, as mentioned in ancient Indian books like Charak Samhita, Mahdhav Nidan and Astang Sanghra, there are about 600 plants, which are stated to have antidiabetic property. Wide arrays of plant derived active principles representing numerous phytochemicals have demonstrated consistent hypoglycemic activity and their possible use in the treatment of diabetes mellitus.

Indian plants which are most effective and commonly studied in relation to diabetes are namely *Allium cepa, Allium sativum, Aloevera, Berberis aristata, Cajanus cajan, Coccinia indica, Caesalpinia bonducella, Cyperus rotundus, Ficus bengalenesis, Gymnema sylvestre, Momordica charantia, Ocimum sanctum, Pterocarpus marsupium, Swertia chirayita, Syzigium cumini Terminalia belerica, Terminalia chebula, Tinospora cordifolia, Trigonella foenum, Phyllanthus emblica, Annona squamosa* etc.

Herbal medicines have been used to cure diabetes as anti-diabetic regimens alone or in compound. Therefore, research is still on the nascent stage to find more effective and safer hypoglycemic agents. For a long time, several medicinal plants have been used for the treatment of diabetes in the form of compound drugs. Moreover, after the reference made by researchers on diabetes mellitus, investigations on the hypoglycemic activity of compound drugs of medicinal plants have been more important.

Polyherbal formulations may enhance the pharmacological activity and reduce the concentrations of single herbs, thereby reducing adverse effects. Plant formulation and combined extracts of plants have been used as a drug rather than individual.

Exploring an effective drug either single or in combination against diabetes is challenging still. Hence we planned to develop antidiabetic polyherbal formulation in the form of capsule containing ethanolic extracts of *Berberis aristata* (dried Stem), *Terminalia chebula* (pericarp of matured fruit), *Emblica officinalis* (pericarp of dried

matured fruit),*Terminalia belerica* (pericarp of dried ripe fruit) and *Cyperus rotundus* (dried rhizome). Ethno medically, the preparation was prescribed in the form of Decoction[17]. This traditional dosage form has several disadvantages like shelf life of decoction as per literature is three hours[18] making it prone to physical, chemical and microbiological instability and it is also bitter in taste. To overcome these problems we planned to prepare ethanolic extract and followed by development of this preparation in to a suitable drug delivery system in the form of capsule was sought to be of appropriate pharmacopoeial quality and would have similar release of the actives as that of the traditional dosage form.

2. REVIEW OF LITERATURE

1. **Muhummaed Rizwan et al., (2017)** repored the Phytochemical and Biological Screening of *Berberis aristata*[19].
2. **Hamsalakshmi et al., (2017)** reported the Pharmacognostical, Phytochemical and anti hyperlipidemic potentials of *Terminalia Belerica*[20].
3. **Srishti Dhyani et al., (2016)** reported the Comparative Pharmacognostical Study of Rhizome of Mustaka and Nagar Mustaka[21].
4. **Sarvesh kumar Bharathi et al., (2016)** reported the Pharmacognostical and Phytochemical Evaluation in *Daruharidra*[22].
5. **Nishant Pathak et al., (2016)** reported the anti-diabetic activity of commercially available extracts of *Phyllanthus emblica* in Streptozocin induced diabetic rats[23].
6. **Alagar raja M et al., (2014)** reported the values of Standardiasation parameters, Pharamacogonstic Study, Preliminary Phytochemical Screening and *In vitro* Ant diabetic ability of *Emblica officinalis* fruits as per WHO guideline[24].
7. **Aji Abraham et al., (2014)** reported Pharmacognostical Studies of the fruits of *Terminalia Belerica* (Gaertn.)[25].
8. **Nidhi Khemaka et al., (2014)** reported preliminary pharmacognostical and pytochemical evaluation of prashata and Aprashata Haritaki (*Terminalia chebula* Retz)[26].
9. **Sri Rajani Sivapalan et al., (2012)** reported Physiochemical and Phyto-chemical studies of Rhizome of *cyperus rotundus* Linn[27].
10. **Disha Arora et al., (2012)** reported Phytochemical and Microscopical investigation on *Emblica officinalis*[28].

11. **Surendra Kumar Sharma et al., (2011)** reported Morphological, Microscopical and Phytochemical investigation on the rhizomes of *cyperus rotundus* Linn[29].

12. **Nitinkumar Upwar et al., (2011)** reported the hypoglycemic Effect Of Methanolic Extract of *Berberis Aristata* Dc Stem on normal and Streptozotocin Induced Diabetic Rats[30].

13. **Sabu MC et al., (2009)** reported antidiabetic and antioxidant activity of *Terminalia belerica.*Roxb[31].

14. **Gandhipuram periyasamy et al., (2006)** reported antidiabetic effect of fruits of *Terminalia chebula* in Streptozotocin induced diabtic rats[32].

15. **Nishikant A et al., (2006)** reported antidiabetic activity of hydro-ethanolic extract of *Cyperus rotundus* in alloxan induced diabetes in rats[33].

3. PLANT PROFILE

Berberis Aristata DC.[35,36]

Fig:8 whole plant of *Berberis Aristata DC.*

Synonym	**: *Berberis Tinctoria, Berberis chitia* Ham**
Family	**:** Berberidaceae
Common name	:Tree turmeric
Parts used	:Dried stem
Vernacular Names	
Sanskrit	:Daruharidra
English	:Tree turmeric
Hindi	: Daruhallada
Tamil	:Maramanjal
PLANT TAXANOMY	
Kingdom	**:**Plantae
Order	**:** Angiosperms
Family	**:** Berberidaceae
Genus	: *Berberis*
Species	: *Berberis aristata*

Fig:9 Dried stem of *Berberis aristata*

Distribution : Himalayas, Nepal.

Description:

Spinous shrubs; axillary thorns with fragrant greenish flowers in racemes and dark red-brown berries.

Phytoconstituents:

Plant contains alkaloids: Berberine, Oxycantine, Berbamine, Palmatine, Jatrorrhisine, columbamine, Berberrubine, Hydrastine, Gum, Starch.

Ethno- Medicinal uses:

Fruits :Haemorrhoids, laxative

Root : Opthalmia, antipyretic, laxative,tonic, malaria fever, rheumatism, diarrhoea, skin diseases.

Stem : Diarrhoea, diabetes, ulcers, opthalmic diseases.

Dose : 5-10 ml of the drug in Deoction form.

Terminalia Chebula Retz.[36,37]

Fig:10 whole plant of *Terminalia Chebula*

Family	**:** Combretaceae
Common name	: Myrobalan
Parts used	: Pericarp of mature fruits
Vernacular Names	
Sanskrit	: Abhaya, Haritaki
English	: Black Myrobalan
Hindi	: Harra, Harad
Tamil	: Kadukkai

PLANT TAXANOMY

Kingdom **:** Plantae

Order **:** Myrtales

Family **:** Combretaceae

Genus **:** *Terminalia*

Species : *Terminalia chebula*

Fig:11 *Terminalia chebula* fruit

Distribution : Throughout the greater part of India

Description:

Deciduous trees, Leaves ovate or elliptic. Flowers yellow or creamy white, in spikes. Fruits 5-ridged.

Phytoconstituents:

Fruits contain- Tannic acid, gallic acid, Chebulinic acid, mucilage,glycoside (Anthroquinone), Carbohydrates, aminoacids,phosphoric acid, succinic acid.

Ethno- Medicinal uses:

Fruits : Diabetes, dysentry, diarrhoea, gout, malaria, sore throat, pneumonia, Anaemia, typhoid

Bark : Ezema

Dose **:** 3-6g of the drug in powder form

Emblica officinalis Gaertn.[38,39]

Fig 12:Whole plant of *Emblica officinalis*

Synonym	**:** *Phyllanthus emblica* L.
Family	**:** Euphorbiaceae
Common name	: Amla
Parts used	: Pericarp of dried matured fruits
Vernacular Names	
Sanskrit	: Amalaka, Dhhatriphala
English	: Emblic myrobalan
Hindi	: Amvala
Tamil	: Nelli, Nellikkai
PLANT TAXANOMY	
Kingdom	**:** Plantae
Order	**:** Malpighiales
Family	**:** Euphorbiaceae
Genus	**:** *Phyllanthus*
Species	: *Phyllanthus emblica*

Fig 13: *Phyllanthus emblica* fruit

Distribution : Throughout India

Description:

Large, deciduous trees, with distichous, linear-oblong leaves. Flowers greenish-yellow, in fascicles on leafless branches. Fruits globose, fleshy; Seeds bony.

Phytoconstituents:

Fruits contain protein, fats, fibres, carbohydrates, vitamin c, Nicotinic acid, Tannins. After drying fruits contain Gallic acid, Ellagic acid, Flavin & glucose. Seed contains Linoleic acid, Linolenic acid, Oleic acid.

Ethno- Medicinal uses:

Fruits : Diabetes, dysentry, diarrhoea, Anaemia, bronchitis, leprosy, leucorrhoea, inflammation of the eyes, malaria, scurvy constipation

Bark : Diarrhoea, gonorrhoea, jaundice.

Leaf : conjunctivitis, diarrhoea, inflammation.

Dose : 3-6g of the drug in powder form

Terminalia belerica (Gaertn.)Roxb.[40]

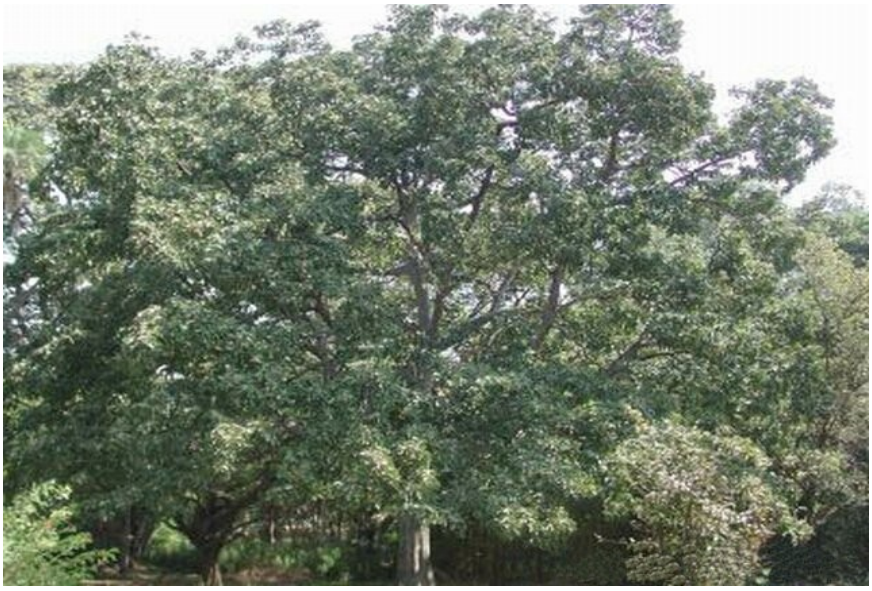


Fig 14: Whole plant of *Terminalia belerica*

Family **:** Combretaceae

Common name : Beleric myrobalan

Parts used : Pericarp of dried ripe fruit

Vernacular Names

Sanskrit : Bibhitaka,Aksha,Vibhita

English : Beleric myrobalan

Hindi : Bahera

Tamil : Thantrikkai

PLANT TAXANOMY

Kingdom **:** Plantae

Order **:** Myrtales

Family **:** Combretaceae

Genus **:** *Terminalia*

Species : *Terminalia belerica*

Fig 15: *Terminalia belerica* fruit

Distribution : Throughout the forest of India

Description:

Deciduous trees, with broadly ovate leaves clustered at the end of branches. Flowers yellow or creamy white, in spikes. Fruits ellipsoid, 5-ridged.

Phytoconstituents:

Fruits contains β-Sitosterol, gallic acid, ellagic acid, chebulagic acid, Galloyl, gluocse, many free sugars, manitol, galactose, rhamnose, glucoside.

Ethno- Medicinal uses:

Fruits : Diabetes, diarrhoea, Anaemia, asthma, bronchitis, leprosy, cough, cardiac diseases, liver problems,headache, constipation, skin diseases, inflammation , malaria, sore throat.

Bark : Anaemia, Leucoderma,cold

Seed oil: Swelling, skin diseases.

Dose : 3-6g of the drug in powder form

Cyperus rotundus L.[41,42]

Fig16 :Whole plant of *Cyperus rotundus*

Synonym	**:** Cyperus hexastachys Rottb.
Family	**:** Cyperaceae
Common name	: Nut Grass
Parts used	: Dried rhizome
Vernacular Names	
Sanskrit	: Musta,Mustaka
English	: Nut Grass
Hindi	: Motha, Nagarmotha
Tamil	: Korai, Korai-Kizhangu
PLANT TAXANOMY	
Kingdom	**:** Plantae
Order	**:** Polaes
Family	**:**Cyperaceae
Genus	**:** *Cyperus*
Species	: *Cyperus rotundus*

Fig 17:Rhizomes of *Cyperus rotundus*

Distribution : Throughout the forest of India

Description:

Sedges, with slender stolons terminated by ellipsoid or globose-ovoid tubers. Leaves not exceeding the clum. Spikes born in terminal corymbs; spikelets dusty brown, 8 to 28 flowered, rachilla winged. Achnes triqutous, brown.

Phytoconstituents:

Rhizome contains Penene, cineol, Linoleic acid, oleic acid, Myristic acid, stearic, glycerol, glycoside cyperene, cyperenone, sugars,gum, carbohydrates.

Ethno- Medicinal uses:

Acrid, anthelmentic, anti- inflammatory, diabetes, expectorant, jaundice, leprosy, scabies, dysmenorrhoea, dyspepsia, flatulence, ulcer, stomach pain.

Dose : 3-6g of the drug in powder form

4. AIM AND OBJECTIVE

AIM

The aim of the present work is to develop a polyherbal anti- diabetic capsules from the selected plant material and evaluate the same.

OBJECTIVE

- To perform the raw material analysis.
- To extract the plant material by continuous Hot Percolation method using ethanol as solvent.
- To formulate and evaluate polyherbal capsule.
- To evaluate the antidiabetic activity by *In vitro and In vivo models.*

.

5. PLAN OF WORK

I. Collection and Authentication

II. Processing of raw materials

III. RAW MATERIALS STANDARDIZATION

- Organoleptic Evaluation
- Microscopical evaluation
- Physico-chemical Evaluation.
  - Loss on Drying
  - Determination of Ash values
    - ✓ Total ash value
    - ✓ Acid insoluble ash value
    - ✓ Water soluble ash value
    - ✓ Sulphated ash value
  - Determination of Extractive values
    - ✓ Water soluble extractive value
    - ✓ Alcohol soluble extractive value
    - ✓ Ether soluble extractive value
- Quantitative Estimation of Heavy metals and Inorganic elements
- Microbial load

IV. PHYTOCHEMICAL STUDIES

- Preliminary phytochemical screening of powder and extracts
- Preparation of Extract
- Fluorescence analysis of raw materials
- Thin Layer Chromatography
- HPTLC - Finger print analysis

V. DEVELOPMENT OF FORMULATION

- Pre formulation studies
  - Selection of excipient

- Flow property measurement
  - Bulk density
  - Tapped density
  - Compressibility index
  - Hausner's ratio
  - Angle of repose
- Trial batches (I, II, III, IV) (Selection of optimized batches)

- Formulation of capsules

VI. STANDARDIZATION OF POLYHERBAL CAPSULES

- Description
- pH
- Uniformity of weight
- Disintegration time
- Ash value
- Extractive value
- Quantitative estimation of Phytoconstituents
- Quantitative Estimation of Heavy metals and Inorganic elements
- Microbial load

VII. PHARMACOLOGICAL STUDIES

- *In vitro* studies:
  - α-amylase inhibitory assay.
- *In vivo* Streptozotocin induced diabetes in rats
  - Blood glucose level
  - Lipid profile

6. MATERIALS AND METHODS

MATERIALS

Polyherbal antidiabetic formulation consists of five herbs viz., *Berberis aristata (*dried Stem), *Terminalia chebula* (pericarp of matured fruit), *Emblica officinalis* (pericarp of dried matured fruit),*Terminalia belerica* (pericarp of dried ripe fruit) and *Cyperus rotundus* (dried rhizome).

Table 1. MATERIALS SELECTED FOR FORMULATION

S.no	Name of the materials	Manufacturer /Supplier	Use in formulation
1	*Berberis aristata*	M/S. K.Ramaswamy Chetty drug dealer,Chennai-03	Active ingredient
2	*Terminalia chebula*	M/S. K.Ramaswamy Chetty drug dealer,Chennai-03	Active ingredient
3	*Emblica officinalis*	M/S. K.Ramaswamy Chetty drug dealer,Chennai-03	Active ingredient
4	*Terminalia belerica*	M/S. K.Ramaswamy Chetty drug dealer,Chennai-03	Active ingredient
5	*Cyperus rotundus*	M/S. K.Ramaswamy Chetty drug dealer,Chennai-03	Active ingredient
6	Lactose	Indian research products limited	Diluent
7	Micro crystalline cellulose	Pharma French Ltd.	Diluent/Disintegrant
8	Magnesium carbonate	Kniss Laboratories	Adsorbant
9	Starch	Alkimas pvt Ltd,Chennai	Binder/Disintegrant
10	Sodium methyl paraben	Global medicines Ltd, Gujarat	Preservative
11	Bronopol	Global medicines Ltd, Gujarat	Preservative

6.1. COLLECTION AND AUTHENTICATION

Herbs used for formulation were procured from the authentic suppliers and further authenticated by Dr. K.N. Sunil Kumar R.O. and HOD Pharmacognosy, Central Siddha Research Institute, Government of India, Arumbakkam, Chennai-106.

6.2. PROCESSING OF RAW MATERIALS

The procured plant materials were cleaned thoroughly. They were then dried under shade for a week or so. Once they were completely dried, they were ground into coarse powder and stored in air tight containers and preserved for the further processing.

6.3. STANDARDISATION OF RAW MATERIALS[43]

Shade dried powdered plant materials of the plants, *Berberis aristata (*dried Stem), *Terminalia chebula* (pericarp of matured fruit), *Emblica officinalis* (pericarp of dried matured fruit),*Terminalia belerica* (pericarp of dried ripe fruit) and *Cyperus rotundus* (dried rhizome) used for the standardization of raw materials.

6.3.1. ORGANOLEPTIC EVALUATION

Organoleptic evaluation defines the majority of information on the identity, purity of the material which are of primary importance for the establishment of degree of quality done by sensory organs for the evaluation of drugs colour, odour, taste and specific characters.

In this study the following organoleptic characters like physical appearance, taste and odour of plant materials were evaluated and confirmed with reference samples.

6.3.2 MICROSCOPICAL EVALUATION

POWDER MICROSCOPY[44]

Powder characters show the detailed examination of a drug which is mainly used to identify the organised drugs by their known structural characters. The

structural characters are distinguished with various reagents and stains. The powder characters of the crude drug powder were studied using microscope.

Methodology

A pinch of the powdered sample was mounted on a microscopic slide with a drop of phloroglucinol and conc. HCl. Characters were observed under microscope.

6.3.3 PHYSICO CHEMICAL EVALUATION

Out of the numerous practical applications of pharmacognosy, the great importance for the pharmaceutical industry is in the evaluation of the crude drugs. The evaluation of these parameters shows the clear idea about the specific characteristic of crude drugs. It is virtually impossible to avoid some naturally occurring inorganic and organic contaminants while collection from soil. The procedures normally adopted to get the purity and standards of a crude drugs which affects the product quality which include the determination of various parameters..

A. LOSS ON DRYING

The test for loss on drying determines both water and volatile matter in the crude drug. The loss on drying test is important when the herbal substances are known to be hygroscopic. An excess of water in herbal materials will encourage microbial growth, presence of fungi, insects and deterioration. In modern pharmaceutical technology, the water content provides information concerning the shelf life and the quality of the drugs. Loss on drying is the loss of mass expressed as % w/w.

About 10 g of drug was weighed in a tarred flat weighing bottle previously dried and dried at 105^{o}C for 5 hours cooled in a suitable dessicator and weighed. The drying was continued and weighed to a constant weight at one hour interval.

$$\text{loss on drying\%} = \frac{\text{Final weight of the sample}}{\text{Initialweight of the sample}} \text{X100}$$

B. DETERMINATION OF ASH VALUES

The ash content of crude drug is generally taken as the residue remaining after incineration. It usually represents the non-volatile inorganic salts like metallic salts and silica naturally occurring in the drug and adhering to it, but it may include inorganic matter added for the purpose of adulteration, contamination and substitution. This is important parameter for the evaluation of crude drugs. The ash value can be determined by three different methods like total ash, acid insoluble ash and water soluble ash. Sulphated ash is also ash value to find out the sulphated residue.

Total ash

Incinerated 2g of the powdered drug in a tared silica crucible at 450ºC in a muffle furnace until carbon completely ashes and ignited to constant weight, removed, cooled in a suitable dessicator for 30 minutes and weighed. Percentage of total ash content was calculated with reference to the air-dried drug.

$$\% \text{ Total ash} = \frac{\text{Weight of residue obtained}}{\text{Weight of sample taken}} \text{X100}$$

Acid insoluble ash

Boiled the ash obtained in total ash for 5 minutes with 25 ml of dilute hydrochloric acid collected the insoluble matter in an ashless filter paper, washed with hot water and ignited at 450ºC to constant weight. Percentage of acid insoluble ash content was calculated with reference to the air-dried drug.

$$\% \text{ Ash insoluble ash } = \frac{\text{Weight of residue obtained}}{\text{Weight of sample taken}} \text{X100}$$

Water-soluble ash

The difference in weight between the total ash and the residue after treatment of the total ash with water.

Determination of Water-soluble ash

To the crucible containing the total ash, add 25 ml of water and boil for 5 minutes. Collect the insoluble matter in a sintered-glass crucible or on an ashless filter paper. Wash with hot water and ignite in a crucible for 15 minutes at a temperature not exceeding 450^0C. Subtract the weight of this residue in mg from the weight of total ash. Calculate the content of water-soluble ash in mg per g of air-dried material.

$$\% \text{ Water soluble ash } = \frac{\text{Weight of residue obtained}}{\text{Weight of sample taken}} \text{X100}$$

Sulphated ash

Heated a silica crucible to redness for 10 minutes, cooled in a dessicator and weighed. 1g of the substance was transferred into the crucible, ignited gently at first, until the substance is thoroughly charred. Cooled and moistened the residue with 1ml of sulphuric acid, heated gently until white fumes are no longer evolved and ignited at 800^oC until all black particles disappeares. Allowed the crucible to cool, added a few drops of sulphuric acid and ignited as before to a constant weight cooled and weighed.

$$\% \text{ Sulphated ash } = \frac{\text{Weight of residue obtained}}{\text{Weight of sample taken}} \text{X100}$$

B. DETERMINATION OF EXTRACTIVE VALUES

The method determines the amount of active constituents in a given amount of crude drugs when extracted with the solvents. The extraction process of crude drug with a particular solvent yields a solution containing different phytoconstituents. The composition of these phytoconstituents provides the preliminary information on the quality of a particular drug sample.

Water soluble extractive

Macerated 5 g of the air dried, coarsely powdered drug, with 100 ml of chloroform water in a closed flask for twenty-four hours, shaking frequently during six hours and allowed to stand for eighteen hours. Filtered and evaporated 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, dried at 105ºC to constant weight and weighed. The percentage of water-soluble extractive value with reference to the air-dried drug was calculated.

$$\%\ \text{Water soluble extractive value} = \frac{\text{Weight of residue obtained after drying}}{\text{Weight of sample taken}} \text{X}100$$

Alcohol soluble extractive

Macerated 5 g of the air dried, coarsely powdered drug, with 100 ml of ethanol (95%) in a closed flask for twenty-four hours, shaking frequently during six hours and allowed to stand for eighteen hours. Filtered and evaporated 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, dried at 105ºC to constant weight and weighed. The percentage of water-soluble extractive value with reference to the air-dried drug was calculated.

$$\text{Alcohol soluble extractive value} = \frac{\text{Weight of residue obtained after drying}}{\text{Weight of sample taken}} \text{X}100$$

Ether soluble extractive

The type of ether soluble extractive values determined for evaluation of crud drugs are volatile and no-volatile ether soluble extractives .the volatile ether soluble represents volatile oil content of the drug, while non-volatile ether – soluble extractives represent resin, fixed oils or colouring matter present in drugs. The precentage of ether soluble extractive was calculated.

$$\text{Ether soluble extractive value} = \frac{\text{Weight of residue obtained after drying}}{\text{Weight of sample taken}} \text{X}100$$

6.3.4. QUANTITATIVE ANALYSIS OF HEAVY METALS[45]

INSTRUMENTATION PARAMETERS:

Instrument name:

Inductive coupled plasma-Optical emission spectroscopy.

Instrument Model:

PE Optima 5300DV ICP-OES; Optical system Dual view-axial or radial.

Detector system:

Charge coupled detector, (UV-Visible detector which is maintaining at -40º C) to detect the intensity of the emission line.

Light source (Torch):

Positioned horizontally in the sample compartment along the central axis of the spectrometer optics. Changing from axial to radial viewing is a simple software command and is accomplished by computer control of a mirror located in the optical path. The torch assembly of this system comprises of two concentric quartz tubes.

Standard alumina injector : 2.00mm inner diameter

Spray Chamber : Scott type

Nebulizer : Cross flow gem tip

Preparation of sample by acid digestion method:

50mg of powder was treated with acid mixture of sulphuric acid: water in the ratio of 4:1in the Kjeldahl flask and heated continuously till the solution is colourless. The sample mixture was then transferred in a 25ml volumetric flask and made up to

the volume with distilled water. Blank solution was prepared as above without sample.

The standards of Arsenic, Lead, Mercury and Cadmium were prepared as per the protocol and the calibration curve was developed for each of them.

Detection:

Samples were analyzed for the detection and quantification of the calcium, sulphate, borate, silver, aluminium, copper, potassium, chloride by Inductively Coupled Plasma Emission Spectrometry.

6.3.5. MICROBIAL LOAD ANALYSIS[46]

The following tests were carried out for the estimation of number of viable aerobic microorganisms present and for detecting the presence of designated microbial species in the herbal medicines.

1. Total aerobic viable count
2. Yeasts and moulds
3. *Escherichia coli*
4. *Clostridia*
5. *Salmonellae*
6. *Shigella*
7. *Pseudomonas*
8. *Staphylococcus*

Pre-treatment of the raw material

The crude raw materials were grinded to dissolve and diluted to 100ml with buffered sodium chloride-peptone solution adjusted the pH to 7.0.

Procedure

For bacteria

Petri dishes of 9–10 cm in diameter was used. To one dish added a mixture of 1ml of the pre-treated raw material and about 15 ml of liquefied casein-soybean digest agar at a temperature not exceeding 45 °C. The material was diluted to obtain an expected colony count of not more than 300. The material was spreaded on the surface of the solidified medium, inverted them and incubated at 30–35°C for 48–72 hours period of time. Counted the number of colonies formed and calculated the results using the plate with the largest number of colonies.

For fungi

Petri dishes of 9–10 cm in diameter was used. To one dish added a mixture of 1ml of the pretreated material and about 15 ml of liquefied Sabouraud glucose agar with antibiotics at a temperature not exceeding 45°C. Spreaded the pretreated material on the surface of the solidified medium in a Petri dish. Diluted the material to obtain an expected colony count of not more than 100 and incubated them upright at 20–25 °C for 5 days. Counted the number of colonies formed and calculated the results using the dish with not more than 100 colonies.

Escherichia coli

1ml of the material was added to 100 ml of MacConkey broth and it was incubated at 43–45°C for 24 hours. A subculture was prepared on a plate with MacConkey agar medium and incubated at 43–45°C for 24 hours. Growth of red, generally non-mucoid colonies of Gram negative rods, sometimes surrounded by a reddish zone of precipitation indicates the presence of *E. coli*.

Clostridium

10ml of the pretreated material was added to two suitable vessels, each containing 100ml of cooked-meat medium, heated just prior to use, to 100°C for a few minutes and cooled to 37°C. To distinguish between sporing and non-sporing

organisms, one vessel was sealed with a layer of sterile paraffin immediately. The other vessel was heated at 65°C for 30 minutes and then similarly sealed. Both vessels were incubated at 35–37°C and examined every 24 hours for up to 4 days. Growth of sporing organisms occurs in the vessel which was heated after inoculation. If no growth occurs in either of the vessels, the sample passes the test for absence of *Clostridia* and other anaerobic bacteria.

Salmonella

The pre-treated plant material or product was incubated at 35–37°C for 5–24 hours, as appropriate for enrichment.

Primary test

10 ml of the enriched culture was transferred to 100 ml of tetrathionate bile brilliant green broth and was incubated at 42–43°C for 24 hours. Subcultures were prepared on two agar media: deoxycholate citrate agar and brilliant green agar. Then, they were incubated at 35–37°C for 24 hours. The well-developed, colourless colonies in deoxycholate citrate agar medium and the small, transparent and colourless, or opaque, pink or white (frequently surrounded by a pink to red zone) colonies in Brilliant green agar medium indicates the presence of *Salmonella* spp.

Shigella

Direct inoculation of agar plates

3 loopful of the pretreated material was transferred to the plates and were incubated at 35–37°C for 24 hours. A general purpose plating medium of low selectivity and one of high selectivity was inoculated. Mac Conkey agar was used as a medium of low selectivity. Mac Conkey agar with 1µg/ml of potassium tellurite was used for *S.dysenteriae* type 1 (Sd1). A

small inoculum was used. It was incubated at 35–37°C for 24 hours. Xylose-lysine-desoxycholate (XLD) agar was used as a medium of high selectivity for isolation of *Shigella*.

Identification of colonies on plating media

Colonies suspicious for *Shigella* will appear as follows:

- Mac Conkey agar: convex, colourless, 2–3 mm
- XLD agar: red, smooth, 1–2 mm

If the colonies found well seperated of typical appearance from each of the plating media it can be taken for further testing by making a mark on the bottom of the petridish.

Inoculation of Triple sugar iron agar (TSI)

Three characteristic colonies from the plating media was picked and inoculated into Triple sugar iron agar (TSI) as follows: stabbed the butt and then streaked the slant with a zigzag configuration. The tubes were properly labelled. It was then incubated overnight. On the following morning, the reactions in the TSI tubes was examined.

Tubes suspicious for *Shigella* will have an acid (yellow) but and an alkaline (red) slant. They will not produce gas (no bubbles or cracks in the agar) and will not produce hydrogen sulfide (no black along the stab line).

Pseudomonas aeruginosa

1ml of the pretreated material was inoculated in 100ml of soybean-casein digest medium. Mixed and incubated at 35–37°C for 24–48 hours. Prepared a subculture on a plate of cetrimide agar and incubated at 35–37°C for 24–48 hours.

If no growth of microorganisms is detected, the material passes the test. If growth of colonies of Gram-negative rods occurs, usually with a greenish fluorescence.

Staphylococcus aureus

Pretreated material was incubated at 35–37 °C for 24–48 hours.

The material passes the test if no growth of microorganisms is detected. Black colonies of Gram-positive cocci often surrounded by clear zones may indicate the presence of *Staphylococcus aureus*.

6.4. PHYTOCHEMICAL STUDIES[47,48]

Herb is a biosynthetic laboratory, which contains chemical compounds such as carbohydrates, proteins and lipids that are utilized as food. It also contains secondary products like glycosides, alkaloids, flavonoids, tannins etc. The detection of these active principles in medicinal plants plays a strategic role in the phytochemical investigation of crude drugs and extracts and is very important in regard to their potential pharmacological effects. These tests facilitate the quantitative estimation and qualitative separation of pharmacologically active chemical compounds and subsequently may lead to the drug discovery and development.

All the plant raw materials were subjected to preliminary phytochemical screening for the detection of various plant constituents.

6.4.1 PRELIMINARY PHYTOCHEMICAL SCREENING

A. Triterpenoids

Salkowski test - Powdered crude drug was treated with few drops of concentrated sulphuric acid, formation of yellow colour indicates the presence of triterpenoids.

B. Flavones

Shinoda test - To the powdered crude drug, few magnesium turnings and few drops of concentrated hydrochloric acid were added and boiled for five minutes formation of red coloration indicates the presence of flavones.

C. Alkaloids

Dragendorff's reagent - To the powdered crude drug, few drops of potassium bismuth iodide solution was added, reddish brown colour indicates the presence of alkaloids.

D. Carbohydrates

Molisch's test - In a test tube containing powdered drug, 2 ml of distilled water and 2 drops of freshly prepared 20% alcoholic solution of α- napthol were added. Mixed well and added 2ml of concentrated sulphuric acid along the sides of the test tube. Formation of red violet ring, which disappears on addition of excess alkali solution, indicates the presence of carbohydrates.

E. Glycosides

Extracted 200 mg of drug with 5 ml dilute sulphuric acid by warming on a water bath, filtered and neutralized the acid extract with 5% solution of sodium hydroxide. 1ml of Fehling's solution A and B were added until it became alkaline (test with pH paper) and heated on a water bath for 2 minutes. Formation of red precipitate was observed, which indicates the presence of glycosides.

F. Phenols

Ferric chloride test - Dissolved a small quantity of the drug with 2ml of distilled water, added a few drops 10% aqueous ferric chloride solution. A blue or green colour was produced, which indicates the presence of phenols.

G. Proteins (Biuret test)

To 1ml of ethanolic extract of the drug, 5 to 8 drops of copper sulphate solution (10%) was added. Formation of violet colour indicates the presence of proteins.

H. Resin

Dissolved a small quantity of the ethanolic extract of the drug with 5 -10 ml of acetic anhydride by gently heating the solution. Cooled and added 0.05 ml of concentrated sulphuric acid. A bright purplish red colour was seen, which rapidly changed to violet indicates the presence of resins.

I. Saponins

To 5 ml of an extract of the drug, a drop of sodium bicarbonate solution was added. Shake the mixture vigorously and left it for 3 minutes. Honey comb like froth developed indicates the presence of saponins.

J. Tannins

The powdered drug was mixed with basic lead acetate solution. Formation of white precipitate, which indicates the presence of Tannins.

K. Steroids

Libermann Burchard's test - The powdered drug was treated with few drops of acetic anhydride, boiled and cooled and added concentrated sulphuric acid from the side of the test tube. A brown ring was formed at the junction two layers and upper layer turns green indicates the presence of steroids.

6.4.2. PREPARATION OF EXTRACT

Extraction is the preliminary step involved in the phytochemical studies. Ethanol proves to the universal solvent. As the present study utilizes a combination of herbs, ethanol was opted for the extraction of the active constituents from the individual plants separately. The method of extraction is Hot Percolation method.

HOT PERCOLATION METHOD

About 200g of coarsely powdered parts of the plant was extracted with Ethanol at 60-70°C. Extract of individual plants were concentrated using rotary vacuum evaporator. The percentage yield, colour and consistency of all the extracts were noted and were taken up for further detailed phytochemical and pharmacological screening.

6.4.3. FLUORESCENCE ANALYSIS [47,48]

In near ultra violet region some of the phytoconstituents shows more or less brilliant colouration when exposed to radiation. The amount of ultra violet light

normally present with visible light is sufficient to produce fluorescence. The phenomenon of emitting visible wavelengths as a result of being excited by radiation of a different wavelength known as fluorescence. This phenomenon make use for the qualitative examination of herbal crude drugs by using ultra violet light.

The presence of constituents with such properties often gives the drug, a characteristic appearance when exposed to ultra violet light, radiation which is reflected as brilliant fluorescence. This fluorescence technique useful for the detection of adulteration in crude drugs.

Raw materials used for the polyherbal capsule preparation were tested for any colour changes under UV light. Samples were tested as such and after treating with organic solvents, alkali and acidic solutions and viewed under ordinary light, short UV (254 nm) and long UV (365 nm) and studied for its fluorescence property.

CHROMATOGRAPHY[49-51]

Chromatographic fingerprinting has been in use for a long time for single chemical entity drug substances. Recently it has become one of the most powerful tools for quality control of herbal medicines. The use of chromatographic fingerprinting for herbal drugs tends to focus on identification and assessment of the stability of the chemical constituents observed by various chromatography techniques such as HPLC, TLC, HPTLC, GC, capillary electrophoresis.

6.4.4. THIN LAYER CHROMATOGRAPHY

Principle

It consists of a thin layer of adsorbent coated on a chromatographic plate, the mobile phase (developing solvent) flows against gravitational force by means of capillary action. The separation is mainly on the differential migration that occurs when the solvent flows along the thin layer of stationary phase. The principle involved thin layer chromatography is adsorption.

Thin layer chromatographic study

The ethanolic extracts of *Berberis aristata, Terminalia chebula*, *Emblica officinalis*, *Terminalia belerica* and *Cyperus rotundus* were subjected to thin layer chromatography (TLC) as per conventional method using silica gel 60F254, 5x3 cm (Merck). Plate markings were made with soft pencil. Glass capillary tubes were used to spot the extract in TLC plates. Different solvent systems ranging from lower to higher polarities were tested for the separation of bioactive components.

In the TLC chamber the solvent system *viz* Butanol: Acetic acid: Aqueous (40:10: 20) were used. After pre-saturation with mobile phase for 30 min the plates were kept inside the chamber and the elution was performed using above mentioned solvent systems. After completion of the elution the plates were dried and subjected to visualized under UV chamber and sprayed using different spray reagents.

R_f values determined by using following formula:

R_f = Distance travelled by the solute / Distance travelled by the solvent.

6.4.5. HPTLC FINGERPRINT PROFILE

HPTLC is one of the advanced and versatile chromatographic technique which helps in the identification of compound and thereby authentication of purity of herbal drugs. It is very quick process. In addition to qualitative detection, HPTLC also provides semi-quantitative information on major active constituents of a drug thus enabling an assessment of drug quality.

HPTLC serves as a convenient tool for finding the distribution pattern of the phytoconstituents which is unique to each plant. The finger print obtained is suitable for monitoring the identity and purity of drugs and for detecting adulteration and substitution. HPTLC technique is helpful in order to check the identity, purity and standardize the quantity of active principles present in the herbal extracts.

Sample Preparation: 1mg of Polyherbal extract was dissolved in 1 ml of methanol.

Developing Solvent System: A number of solvent systems were tried, for extract. The satisfactory resolution obtained for the phytochemical constituent alkaloid was in the solvent butanol-acetic acid-water (4:1:5).

Chromatography: Chromatography was performed on silica gel 60 F254 TLC pre-coated plates using Hamilton syringe and CAMAG LINOMAT 5 instrument. 2 µl of standard solution and 2 µl of the test solution (extract) were loaded as 5 mm band length in the 4×10 glass plates, with the help of a CAMAG LIWOMAT 5 sample applicator at the distance of 10 mm from the edge of the plates.

Development of Chromatogram: After the application of sample, the chromatogram was developed in Twin trough glass chamber 10x10 cm saturated with previously equilibrated mobile phase for 30 minutes. The chromatographic conditions were previously optimized to active the best resolution and peak shape.

Detection of Spots: The air-dried plates were viewed in ultraviolet radiation to mid-day light. The chromatograms were scanned by densitometer at 420 nm. Quercetin was used as the reference standard. The plates were kept in Photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images at White light, UV 254 nm. After scanning the plates the Peak table, Peak display and Peak densitogram were recorded. The retention factor (Rf) was calculated by the WinCats software.

6.5. DEVELOPMENT OF FORMULATION

The ethanolic extracts of *Berberis aristata, Terminalia chebula*, *Emblica officinalis*, *Terminalia belerica* and *Cyperus rotundus* were subjected to freeze drying process .The extracts were dried for a period of time according to their rate of drying . The lyophiliser was made utilized in the Pharmaceutics Laboratory, of our college. Diluents like, Microcrystalline cellulose, Magnesium stearate, Lactose,starch were dried.

All active ingredients were weighed according to the formula, mixed with adsorbent magnesium carbonate followed by diluents and preservatives like Sodium methyl paraben and bronopol as specified in formula were mixed well. The mixture

was blended thoroughly for 30 minutes. Then the powder was transferred to the polythene bags and labelled for further studies.

Table 2. PROPOSED STRENGTH OF FORMULATION

S.NO.	ACTIVE INGREDIENTS	STRENGTH (in mg)
1	*Berberis aristata*	50
2	*Terminalia chebula*	100
3	*Emblica officinalis*	120
4	*Terminalia belerica*	120
5	*Cyperus rotundus*	80

6.5. 1. PREFORMULATION STUDIES[52]

Prior to formulation, it is essential that fundamental physical and chemical properties of the drug molecule and other derived properties of the drug powder are determined. This information decides many of the subsequent events and approaches in formulation development. This first learning is known as Preformulation. It aims to optimize the process of turning a drug into a drug product. During preformulation the physiochemical properties of the drug candidate are determined.

Definition

Preformulation involves the application of biopharmaceutical principles to the physicochemical parameters of drug substance are characterized with the goal of designing optimum drug delivery system.

Before beginning the formal preformulation programs the manufacturing scientist must ensure the following factors:

- The amount of drug available

- The physicochemical properties of the drug already known.
- Therapeutic category and anticipated dose of compound.
- The nature of information, a formulation should have or would like to have.

SELECTION OF THE EXCIPIENTS[53]

The majority of materials filled in the capsules are formulated as powders that are typically mixtures of the active ingredients together with a combination of different types of excipients. This is so because placing powders into hard gelatin capsules often lead to improved bioavailability owing to a higher porosity of the filled mass and the requirements for powder flow is less stringent for filling capsules than for tabletting. Normally, there are three types of excipients used in powder filled capsules i.e. diluents, glidants and lubricants.

Diluents:

Diluents/Fillers are added where the quantity of active ingredient is less (or) difficult to filling. Common tablet/capsule filler include Lactose, Dicalcium phosphate, Microcrystalline cellulose, etc.

Lubricants:

They reduce friction during the filling process. In addition, they aid in preventing adherence of capsule material. Magnesium Stearate, Stearic acid, Hydrogenised vegetable oils and talc are commonly used lubricants.

Glidant:

It is used to improve flow of the powder materials by reducing the friction between the particles. The most effective glidants are the Colloidal silicon dioxide, Talc and Starch.

Preservatives:

The preservatives are added to herbal formulation to prevent contamination, deterioration and spoilage by bacteria, fungal and other microorganisms. The most effective preservatives are the sodium methyl paraben, sodium propyl paraben, sodium benzoate and bronopol.

FLOW PROPERTY MEASUREMENTS[54,55]

The flow property of the blended powder is an important parameter to be measured since it affects the uniformity of dose. It was assessed by the following parameters.

- Bulk density
- Tapped density
- Compressibility index
- Hausner's ratio
- Angle of repose

Bulk density (ρb)

It is determined by measuring the volume of a known mass of powder sample that has been passed through a screen into a graduated cylinder or through a volume measuring apparatus into a cup. It is expressed in g/ml and is given by,

$$\rho b = M/Vo$$

Where, M - is the mass of powder

Vo- is the bulk volume of the powder.

The inter particle interactions that influence the bulking properties of a powder are also the interactions that interfere with powder flow, a comparison of the bulk and tapped densities can give a measure of the relative importance of these interactions in

a given powder. Such a comparison is often used as an index of the ability of the powder to flow.

Tapped density (ρt)

It is achieved by mechanically tapping a measuring cylinder containing a powder sample. After observing the initial volume, the cylinder is mechanically tapped and volume readings are taken until little further volume change is observed.The mechanical tapping is achieved by raising the cylinder and allowing it to drop under its own weight at a specific distance.The tapped volume was measured by tapping the powder to constant volume. It is expressed in g/ml and is given

$$\rho t = M/Vt$$

Where, M - Mass of powder and Vt- Tapped volume of the powder

Compressibility index: (CI)

Compressibility is the ability of powder to decrease in volume under pressure. Compressibility is a measure that obtained from density determination. Weighed quantity of granules was transferred to 50 ml graduated cylinder, volume occupied by granules was noted down. Then cylinder was subjected to 500/ 750 and 1250 taps. The difference between two tabs should be less than 2%. The percentage Compressibility Index is calculated by using formula.

$$CI = Vo\text{-}Vi \times 100/Vo$$

Where, Vo- Untapped density; Vi- Tapped density

Hausner's Ratio

It is measurement of frictional resistance of the granular material. The Ideal range should be 1.2 -1.5, it was determined by the ratio of tapped density and bulk density. **Hausner's Ratio = Vi / Vo**

Where, Vo -Untapped density, Vi -Tapped density

Angle of repose

The tangent of angle of repose is equal to the coefficient of friction between the particles. Hence the rougher and more irregular the surface of particles, the greater will be angle of repose. For determination of angle of repose (θ), the blends were poured through the walls of a funnel which was fixed at a position such that its lower tip was at a height of exactly 2.0 cm above a hard surface. The drug or the blends were poured till the time when upper tip of the pile surface touched the lower tip of the funnel. Angle of repose was calculated using following equation.

$$\textbf{Tan } \theta = \textbf{h/r,}$$

$$\theta = \textbf{tan-1 (h/r)}$$

Where, θ - angle of repose, h- height in cm and r- radius in cm.

Based on the Angle of repose, Compressibility index and Hausner's ratio, the flow property of the granules can be characterized.

Table 3:Angle of Repose, Compressibility Index and Hausner's Ratio

Flow property	Angle repose	Compressibility Index	Hausner's ratio
Excellent	25-30	<10	1.00-1.11
Good	31-35	11-15	1.12-1.18
Fair	36-40	16-20	1.19-1.25
Passable	41-45	21-25	1.26-1.34
Poor	46-55	26-31	1.46-1.59
Very poor	56-65	32-37	1.46-1.59
Very very poor	>66	>38	>1.60

DEVELOPMENT OF FORMULATION -TRIAL BATCHES

Four trial batches of capsules were formulated by varying the composition of the excipients proportions for excellent flow property.

Table 4. DEVELOPMENT OF FORMULATION

S.NO	MATERIALS	TRIAL-1 (mg)	TRIAL-2 (mg)	TRIAL-3 (mg)	TRIAL-4 (mg)
1	*Berberis aristata*	50	50	50	50
2	*Terminalia chebula*	100	100	100	100
3	*Emblica officinalis*	120	120	120	120
4	*Terminalia belerica*	120	120	120	120
5	*Cyperus rotundus*	80	80	80	80
6	Lactose	35	40	55	35
7	Micro crystalline cellulose	18	20	25	10
8	Magnesium carbonate	1.2	3	4.5	5
9	Starch paste	Aq	Aq	Aq	Aq
10	Sodium methyl paraben	0.5	0.5	0.5	0.5
11	Bronopol	0.5	0.5	0.5	0.5

The blended powder of all four trial batches were analysed for its flow characteristics like bulk density, tapped density, compressibility index, Hausner's ratio and Angle of repose.

From the above trial batches, the trial batch 4th trial was found to be the perfect batch and it was selected for the consideration of further studies.

Table :5 Final Batch

S.NO	MATERIALS	TRAIL IV(mg)
1	*Berberis aristata*	50
2	*Terminalia chebula*	100
3	*Emblica officinalis*	120
4	*Terminalia belerica*	120
5	*Cyperus rotundus*	80
6	Lactose	35
7	Micro crystalline cellulose	10
8	Magnesium Carbonate	5
9	Starch paste	Aq
10	Sodium methyl paraben	0.5
11	Bronopol	0.5

6.5.2. FORMULATION OF CAPSULES[54-57]

Capsule is the most versatile of all dosage forms. Capsules are solid dosage form in which one or more medicinal and inert ingredients are enclosed in a small shell usually made of gelatin. There are two types of capsules, “hard” and “soft”. The hard capsule is also called “two piece” as it consists of two pieces in the form of small cylinders closed at one end, the shorter piece is called the “cap” which fits over the

open end of the longer piece, called the “body”. The soft gelatin capsule is also called as “one piece”. Capsules are available in many sizes to provide dosing flexibility.

SELECTION OF CAPSULE SIZE [54,55]

The volume of material to be filled into the capsule should be determined. Generally, capsules of sizes “0” to “4” were readily available in the market and the relationship between the capsule size and related body volume to be known at the development stage. For pharmaceutical products it is unusual to use a size larger than “0” because of the difficulty in swallowing larger size capsules, whilst size “5” is rarely used due to difficulties in the automatic filling process. Capsule of size “0” were selected to fill the polyherbal formulation.

CAPSULE FILLING

- The formulated granules were filled in"0" size capsules to an average net content weight 520mg per capsule by manual filling method.
- The capsules were then dedusted, transferred into polythene bags, labelled and the samples were evaluated as per testing requirements.

6.6. STANDARDISATION OF POLYHERBAL CAPSULES [58]

The developed polyherbal capsules were standardised for its Description, uniformity of weight, disintegration time, moisture content, pH, physicochemical parameters, phytochemical studies, fluorescence analysis, heavy metal analysis and microbial load analysis. Determination of uniformity of weight, disintegration time and moisture content of capsules were carried out as per Indian pharmacopoeial procedures.

6.6.1. DESCRIPTION

The general appearance of a capsule, its visual identity and overall “elegance” is essential for consumer acceptance. The color, shape, odor, surface texture and legibility of any identifying marking are all noted for the capsules prepared.

6.6.2. pH

The pH value of a solution was determined potentiometrically by means of a digital pH meter. The pH meter was calibrated using buffers of 4, 9 and 7 pH. 1g of capsule content was taken and dissolved in 100ml demineralized water. The electrodes were immersed in the solution and the pH is measured.

6.6.3. AVERAGE WEIGHT & UNIFORMITY OF WEIGHT

20 individual units were selected at random and this content was weighed and their average weight was calculated. Not more than two of the individual weights deviate from the average weight by more than the percentage shown in the table and none deviates by more than twice that percentage.

Table 6. SPECIFICATION OF AVERAGE & UNIFORMITY OF WEIGHT

S.NO	DOSAGE FORM	AVERAGE WEIGHT	% DEVIATION
1.	Capsules	<300mg >300 mg	10% 7.5%

6.6.4. DISINTEGRATION TIME

This test is done to measure the time taken by the drug to disintegrate in the body. This is done to determine whether the capsule disintegrates within the prescribed time when placed in a liquid medium under the prescribed experimental conditions. One each capsule was added to each of the six tubes of the basket and a disc was added to each of the tube. The tubes were dipped in 0.1N HCl solution maintained at 37°C.

6.6.5. PHYSICOCHEMICAL PARAMETERS [43]

Physicochemical parameters like loss on drying, ash and extractive values were carried out as per pharmacopoeial procedures as discussed in earlier.(6.4.3)

6.6.6. PHYTOCHEMICAL STUDIES

I. QUALITATIVE PHYTOCHEMICAL ANALYSIS were carried out to detect the presence of phytoconstituents in the polyherbal formulation. They were discussed in earlier chapter[47,48](6.4.4)

6.6.7. II. QUANTITATIVE ESTIMATION OF TOTAL PHENOLIC, FLAVONOID AND TANNINS CONTENT[59,60,61]

ESTIMATION OF FLAVONOIDS

The total flavonoid content is usually determined spectrophotometrically using Ultra-violet spectroscopy.

Preparation of Standard Stock solution

Accurately weighed 25 mg of Quercetin standard transferred to 100 ml of volumetric flask and dissolved with dimethyl sulfoxide(DMSO). The serial dilution (12.5µg/ml to 200µg/ml) were made with dimethyl sulfoxide.

Preparation Of Test Solution

The Polyherbal formulation was weighed accurately equal to the weight of Standard Quercetin and transferred to 100 ml volumetric flask and the extract dissolved with dimethyl sulfoxide (DMSO). The serial dilution (12.5µg/ml to 2005µg/ml) were made with dimethyl sulfoxide.

PROCEDURE

From the prepared solution of standard and test solutions 2ml was withdrawn from each concentration to the test tube and added equal volume of 2% Aluminium Chloride solution to every single concentration. Incubate the solution about 10 minute

at ambient temperature. After 10 minute, measure the absorbance spectrophotometrically at 435 nm with the standard and test sample solutions.

ESTIMATION OF PHENOLIC CONSTITUENTS

Total phenolic content of the extracts were determined using Folin – Ciocalteu's assay. 0.5ml extract solutions were mixed with 2.5ml of 10 fold diluted Folin Ciocalteu's reagent and 2.5ml of 7.5% sodium carbonate. After incubation at 40°c for 30 minutes, the absorbance of the reaction mixtures was measured at 765nm in a spectrophotometer. Three replicates were made for each test sample. Gallic acid was used as a standard and total phenolic content of the extract was expressed in mg of Gallic acid equivalents (mg GAE/g extract)

ESTIMATION OF TANNIN CONTENT

The tannin content was determined by using $FeCl_3$ and gelatin tests 0.1g of the extracts were transferred to a 100ml flask, 50ml of water was added and boiled for 30min. After filtration with cotton filter, filtrate was transferred to a 500ml flask and the volume was made up to the mark with distilled water. 0.5 ml aliquots were transferred to vials, 1ml of 1% $K_3Fe(CN)_6$and 1 ml of 1% $FeCl_3$ were added and the volume was made up to 10ml with distilled water. After 5 min absorbance was measured at 510nm against a reagent blank spectrophotometer and concentration of tannins in the test sample was determined and expressed as mg equivalent of tannic acid per gram of sample.

Quantitative analysis of Heavy metal

The formulation was analyzed for its heavy metals as discussed earlier (6.3.4)

MICROBIAL LOAD ANALYSIS

For the safe use of poly herbal capsules microbial count were done and checked whether total Total aerobic viable count,Yeasts and moulds were within the prescribed limits and the microorganisms *Escherichia coli, Clostridia, Salmonellae,*

Shigella, Pseudomonas, Staphylococcus were absent in the finished formulation. The procedure was discussed earlier (6.3.5)

6.7. PHARMACOLOGICAL EVALUATION

The protocol for conducting the *in vivo* study in female adult albino wistar rats was approved by the Institutional Ethical Committee (IEC) of the Madras Medical College, Chennai-600003, CPSEA registration number 1917/ReBi/16/cpcea/25.10.2016 vide Roc. No.13/AEL/IAEC/MMC,Date:12.09.2017.

6.7.1.*IN VITRO* ANTI – DIABETIC ACTIVITY[62]

α-amylase inhibition assay

α-amylase was dissolved in phosphate buffer saline (PBS, 0.02 mol/L, pH 6.8) at a concentration of 0.1 mg/mL. Various concentrations of sample solutions (0.25 mL) were mixed with α-amylase solution (0.010 mL) and incubated at 37 °C for 5 min. Then the reaction was initiated by adding 0.1 mL 1.0% (w/v) starch substrate solution to the incubation medium. After incubation at 37 °C for 3 min, the reaction was stopped by adding 1 mL DNS reagent (1% Dinitrosalicylic acid, 0.05% Na_2SO_3 and 1% NaOH solution) to the reaction mixture and boiling at 100 °C for 5 min. After cooling to room temperature, the absorbance (Abs) at 540 nm was recorded by a spectrophotometer. The inhibition percentage was calculated by the following equation:

$$\textbf{Inhibition (\%)} = [(Abs1 - Abs2)/Abs1] \times 100$$

where, Abs1=sample and Abs2 = control.

6.7.2. *IN VIVO* ANTIDIABETIC ACTIVITY

ACUTE TOXICITY STUDY[63,64]

The acute toxicity study was designed as per the OECD Guideline for Testing of Chemicals, Acute Oral Toxicity (Acute Toxic Class Method), Guideline 423.

Principle and purposes

Acute toxicity testing determines the toxicity of a chemical or drug substances after single administration. The test is based on stepwise procedure with use of minimum number of animals per step; sufficient information is obtained on the acute toxicity of the test substance to enable its classification. The substance was administered orally to a group of experimental animals at one of the defined dose. The substance was tested using a stepwise procedure, each step using three animals of a single sex. Absence or presence of compound-related mortality of the animals dosed at one step was used to determine the next step, i.e.

- No further testing is needed
- Dosing of three additional animals, with the same dose
- Dosing of three additional animals at the next higher or the next lower dose levels

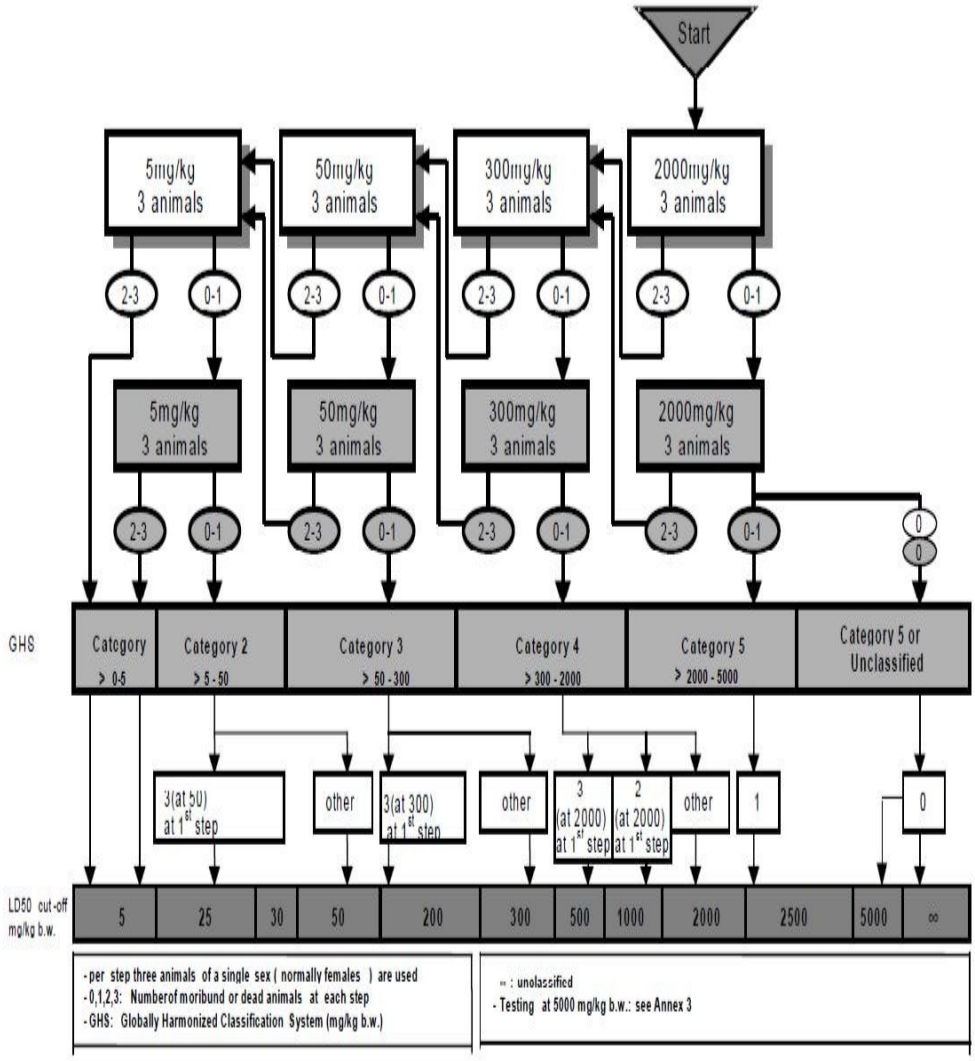
Experimental animals

Healthy adult non pregnant female wistar albino rats weighing between 150-200g were selected for the study. For all the three animals water was provided ad libitum and food was withheld overnight prior to dosing.

Selection of dose levels and administration of doses

Being a traditional herbal medicine, the mortality was unlikely at the highest starting dose level (2000mg/kg body weight). Hence a limit test at one dose level of 2000mg/kg body weight was conducted in all the three animals.

TEST PROCEDURE WITH A STARTING DOSE OF 2000 mg/kg BODY WEIGHT(OECD/OCDE 423)



Test procedure with a starting dose of 2000 mg/kg body weight (OECD/OCDE 423)

Observation

The animals were observed individually after dosing once during the first 30 minutes, periodically for the first 24 hours, with special attention given during the first 4 hours, and daily there-after, for a total of 14 days. The following clinical observations were made and recorded.

Toxic signs

All the rats were observed for any toxic signs.

Pre-terminal deaths

All the rats were observed for any pre-terminal deaths

Body weight

Individual body weight was recorded for all the animals once in a week

Cage side observation

The faeces color, faeces consistency, changes in skin, fur and eyes of the animals were observed once in a week.

Physical examination

Physical observation were observed once in a week.

ANTIDIABETIC EFFECT OF HERBAL FORMULATION IN STREPTOZOTOCIN AND NICOTIMANIDE INDUCED DIABETIC RATS[65-71]

The *Albino Wistar* rats were divided into four different Groups of six animals each as follows.

Group I **:** Diabetic rats treated with Distilled water

Group II **:** Diabetic rats treated with PHF (200 mg/kg)

Group III **:** Diabetic rats treated with PHF (400 mg/kg)

Group IV **:** Diabetic rats treated with Glibenclamide (0.25 mg/kg).

Table: 7 Animal Experimental Design

S. No	Group	Name of the drug	Dose	No of animals	Duration of dosage (days)
1	Group –1	Distilled Water	2ml p.o	6	28
2	Group –2	Polyherbal formulation	200mg/kg p.o	6	28
3	Group – 3	Polyherbal formulation	400mg/kg p.o	6	28
4	Group – 4	Standard (glibenclamide)	0.25mg/kg p.o	6	28

Diabetes was induced in overnight fasted rats by administering single intraperitoneal (i.p.) injection of freshly prepared Streptozotocin (STZ) 50 mg/kg body weight, followed by 120 mg/kg of Nicotimanide (NIC) in 0.1 M citrate buffer (pH 4.5) in a volume of 0.5 ml/kg body weight. Diabetes was confirmed in the STZ + NIC treated rats by measuring fasting blood glucose levels after 48 hrs of induction. After 24 hrs of STZ + NIC injection, the rats were given 5% w/v of glucose solution (2 ml/kg body weight) to prevent hypoglycemic mortality.

Rats with fasting blood glucose of more than 200 mg/dl were considered as diabetics and they were divided randomly into four different groups. The standard (glibenclamide) and herbal formulation were suspended in distilled water and administered once daily through oral gavage for 28 consecutive days. Weekly body weight variations were monitored for all the experimental animals.

BIOCHEMICAL ESTIMATION

The blood samples were collected on initial, 7th, 14th and 21st and 28th days of the treatment, through the tail vein of rats by pricking and were immediately used for the estimation of blood glucose with a glucometer.

At the end of the experiment, the blood sample was withdrawn from all the experimental animals through retro orbital plexus puncture for biochemical analysis.

Statistical Analysis

All the data expressed as mean ± SEM were analysed by Oneway Analysis of Variance(ANOVA) followed by Dunnett's t test for multiple comparison. The statistical analyses were performed using Graphpad Prism version 7.04 of computer software. P values <0.05 were considered as statistically significant.

7. RESULTS AND DISCUSSION

7.1. RAW MATERIAL STANDARDIZATION

7.1. 1. ORGANOLEPTIC EVALUATION

Table : 8 ORGANOLEPTIC CHARACTERS

S.NO	NAME OF THE PLANT	NATURE	COLOUR	ODOUR	TASTE
1	*Berberis aristata*	Coarse powder	Pale Yellowish brown	Odourless	Bitter
2	*Terminalia chebula*	Coarse powder	Dull Yellow	Odourless	Astringent, slightly bitter
3	*Emblica officinalis*	Coarse powder	Grey to black	Odourless	Sore and Astingent
4	*Terminalia belerica*	Coarse powder	Dark brown to black	None	Astringent
5	*Cyperus rotundus*	Coarse powder	Dark black	Pleasant	Pleasant

7.1.2. MICROSCOPICAL EVALUATION

Table 9. POWDER MICROSCOPY

S.NO	PLANT NAME	OBSERVATION
1	*Berberis aristata* (dried stem)	Presence of **Non-lignified elements:** Fibres, stone cells, Thick walled fibres, Parenchyma with crystals. **Lignified elements:** Cork cells in oblique view, Pitted vessel fragment, Fibre fragment, Vessel, Tracheid, Sclereid.
2.	*Terminalia chebula* (pericarp of fruit)	Presence of **Non-lignified elements:** Epicarp, Thick walled parenchyma, **Lignified elements:** Different types of sclereids, Fibro-sclereids, Pitted tracheids.

3.	*Emblica officinalis* (pericarp of fruit)	Presence of **Non-lignified elements:** Epicarp, Thick walled parenchyma, Fibre, Parenchyma.**Lignified elements:** Sclereids of different types
4.	*Terminalia belerica* (pericarp of fruit)	Presence of **Non-lignified elements:** Trichome, Epicarp, Parenchyma with tannin, Tracheids, Vessels. **Lignified elements:** Vessels, Sclereids, Fibro-sclereid
5.	*Cyperus rotundus* (dried rhizome)	Presence of **Non-lignified elements:** Cork, Aerenchyma from root, Tracheidal fibres, Vessels. **Lignified elements:** Group of sclereids, Parenchyma.

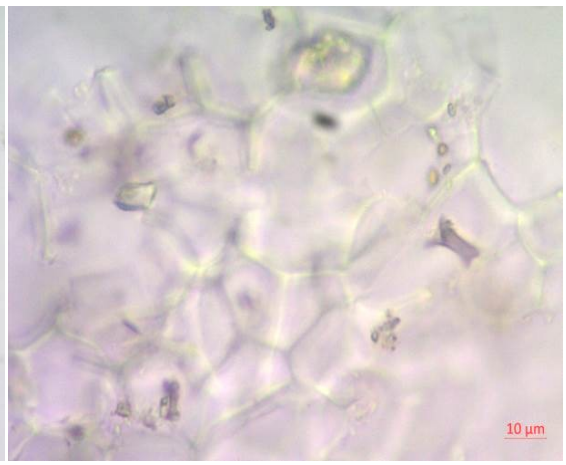
Figure:18. Characters from *Berberis aristata* DC. - Dried stem

18.1 Non-lignified elements

18.1.1 Fibres

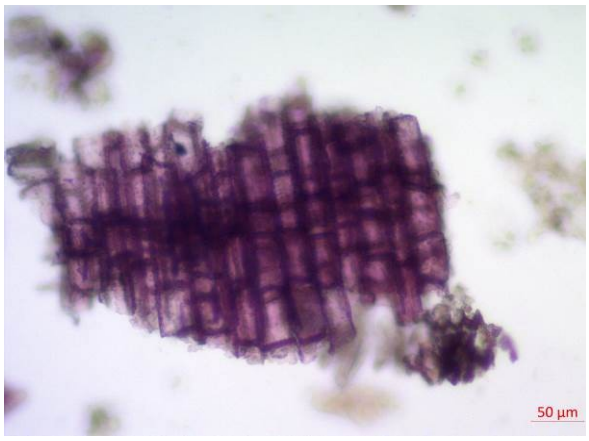
18.1.2 Stone cells

18.1.3 Thick walled fibres



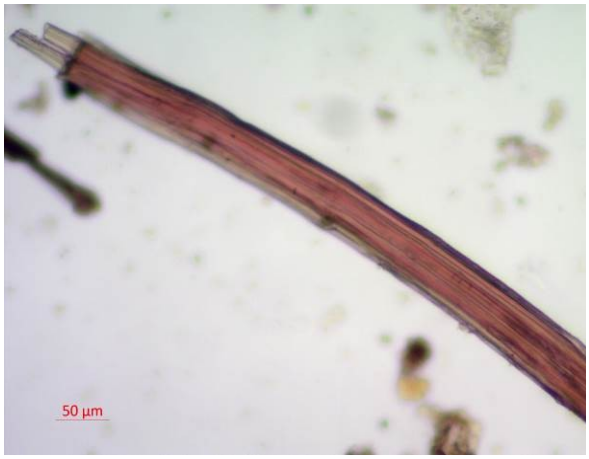
18.1.4 Parenchyma with crystals

18.2 Lignified elements



8.2.1 Cork cells in oblique view

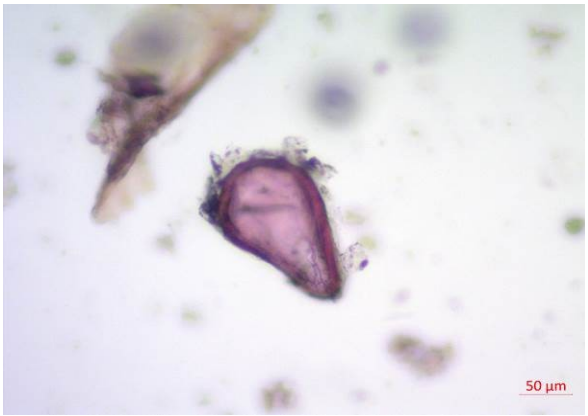
8.2.2 Pitted vessel fragment



8.2.3 Fibre fragment

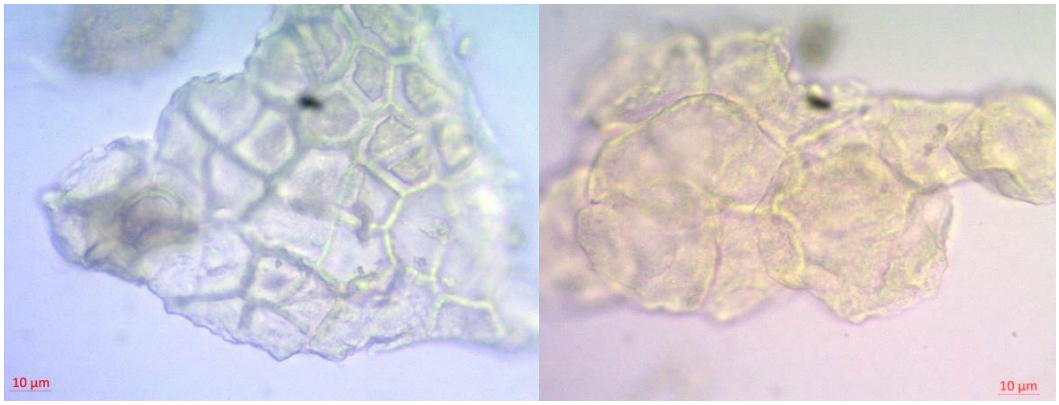
8.2.4 Vessel

8.2.5 Tracheid



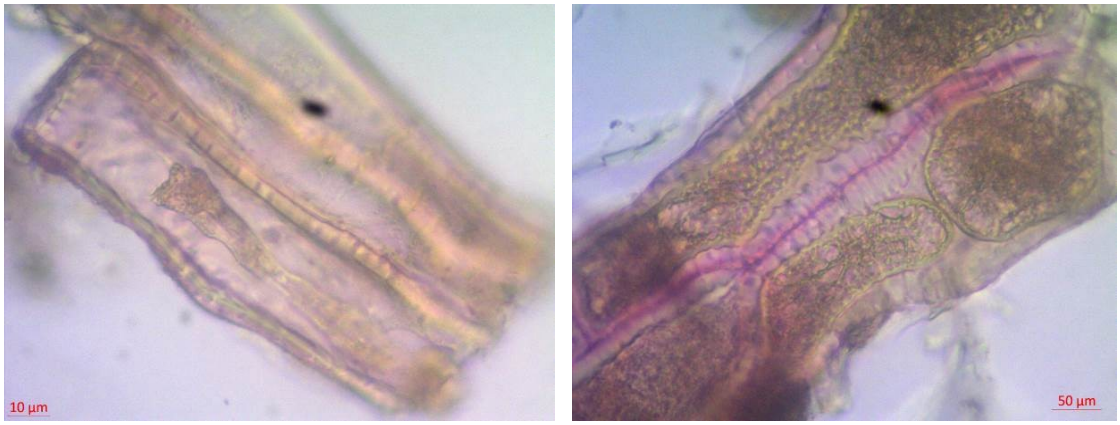
8.2.6 Sclereid

Figure: 19 Characters from *Terminalia chebula* (Gaertn.) Retz. - fruit
19.1 Non-lignified elements



19.1.1 Epicarp **19.1.2** Thick walled parenchyma

19.2 Lignified elements



19.2.1 Different types of sclereids

19.2.2 Fibro-sclereids **19.2.3** Pitted tracheids

nchyma

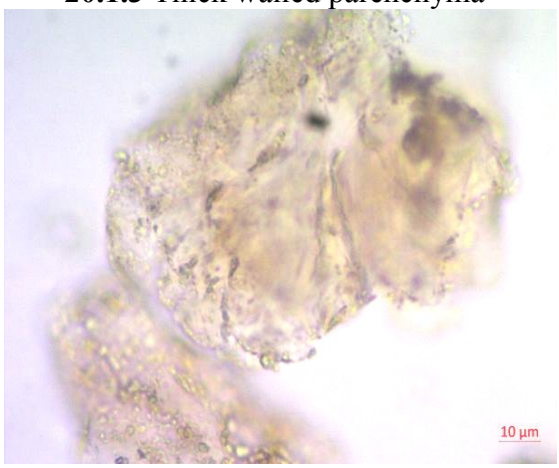
Figure.20 Characters from *Emblica officinalis* Gaertn. – pericarp

20.1 Non-lignified elements

20.1.1 Epicarp

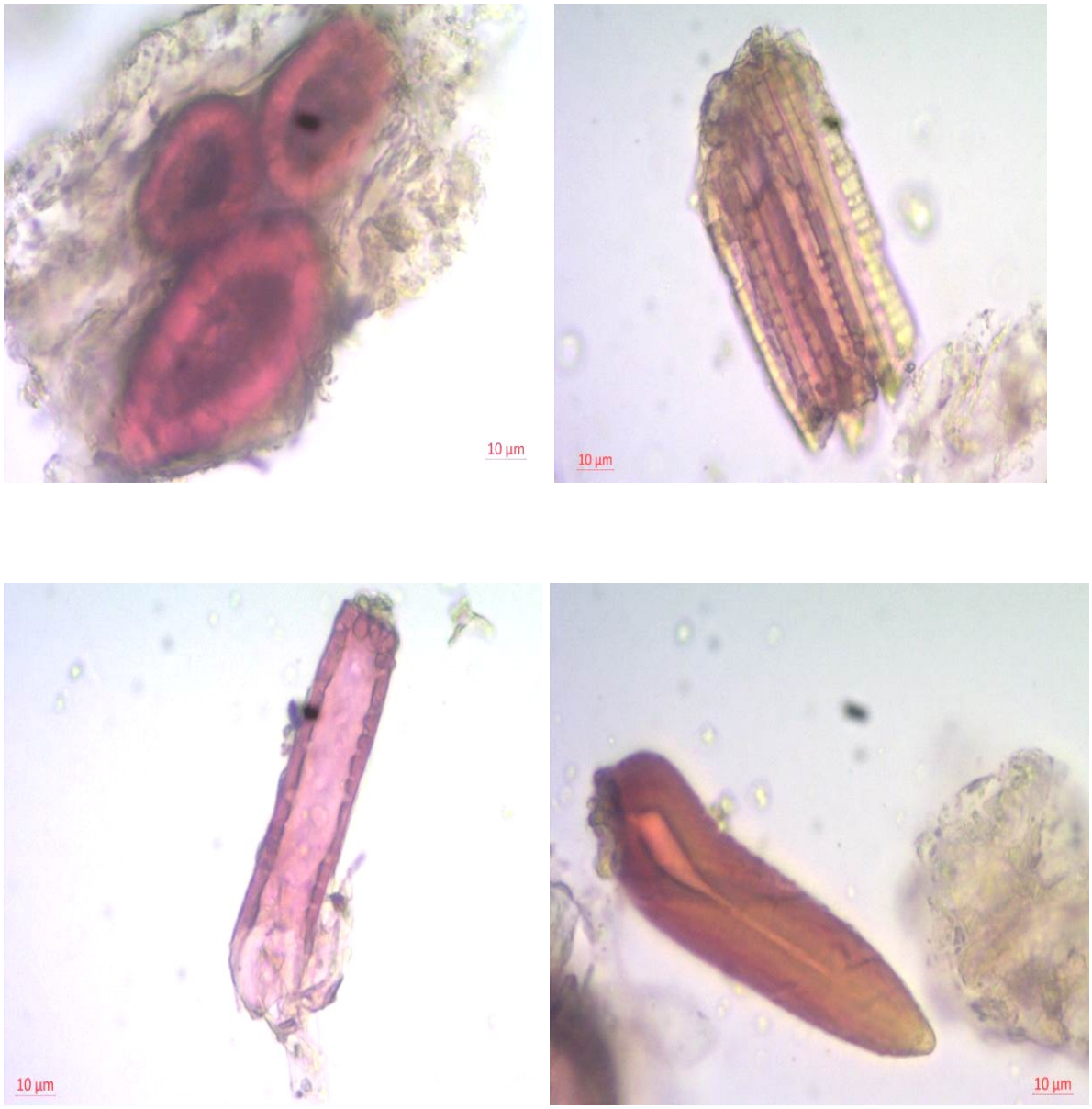
20.1.3 Thick walled parenchyma

20.1.4 Fibre



20.1.5 Parenchyma

20.2 Lignified elements

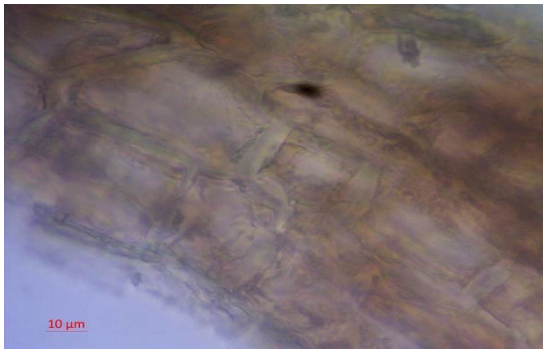


20.2.1 Sclereids of different types

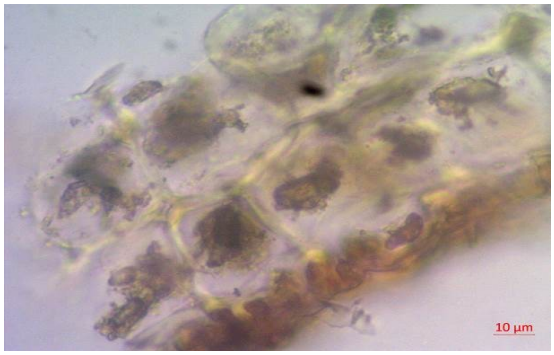
Figure:21 Characters from *Terminalia bellerica* (Gaertn.) Roxb. - fruit

21.1 Non-lignified elements

21.1.1 Trichome



21.1.2 Epicarp



21.1.3 Parenchyma with tannin

21.1.4 Tracheids

21.1.5 Vessels

21.2 Lignified elements

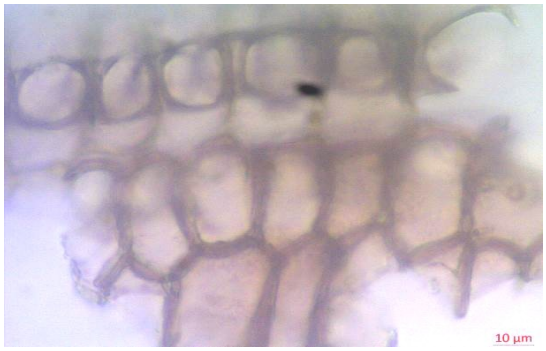
21.2.1 Vessels

21.2.2 Sclereids

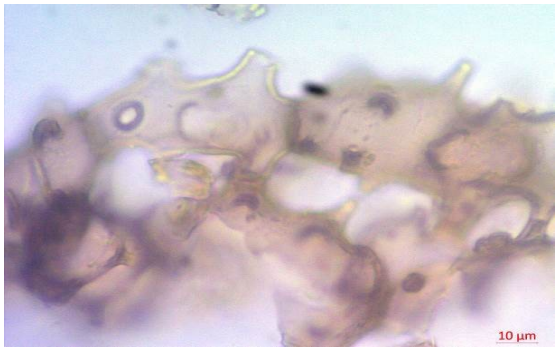
21.2.3 Fibro-sclereid

Figure: 22 Characters from *Cyperus rotundus* L. - rhizome

22.1 Non-lignified elements

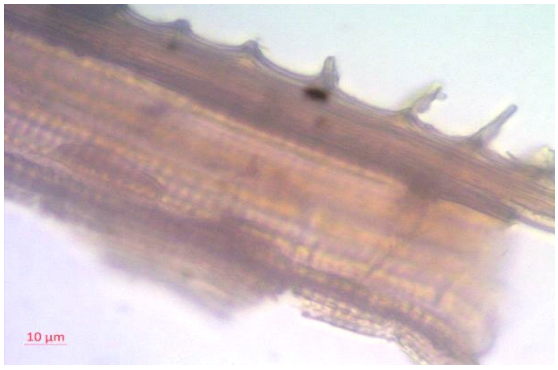


22.1.1 Cork



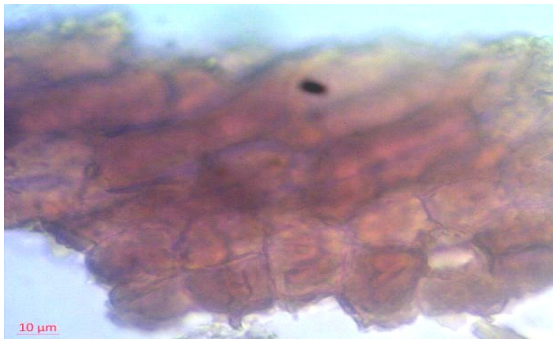
22.1.2 Aerenchyma from root

22.1.3 Tracheidal fibres

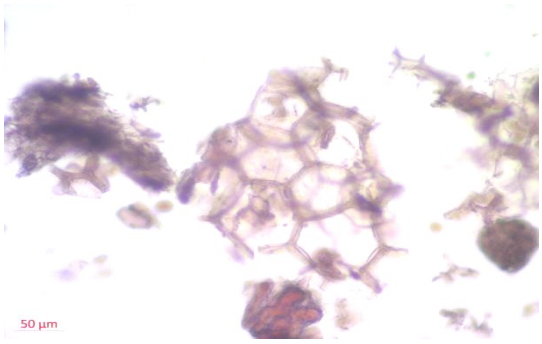


22.1.4 Vessels

22.2 Lignified elements



22.2.1 Group of sclereids



22.2.2 Parenchyma

7.1.3. PHYSIO -CHEMICAL EVALUATION

LOSS ON DRYING

Loss on drying for the raw materials were done. The results obtained and the standared values are given in table.

Table :10 Loss on drying

S.NO	PLANT NAME	LOD (% w/w)	ACCEPTABLE LIMITS(w/w %)
1	*Berberis aristata*	2.74±0.59	NMT 8
2	*Terminalia chebula*	3.67±0.12	NMT 8
3	*Emblica officinalis*	4.61±0.14	NMT 6
4	*Terminalia belerica*	4.16±0.07	NMT 5
5	*Cyperus rotundus*	4.25±0.01	NMT 5

The value are expressed as mean ± SD, (n=3); NMT-Not more than

DETERMINATION OF ASH VALUES

TOTAL ASH CONTENT

Total ash content of raw materials was determined, the values obtained and their acceptable limits defined are given table in table.

Table:11 Total ash value

S.NO	PLANT NAME	TOTAL ASH (% w/w)	ACCEPTABLE LIMITS(w/w %)
1	*Berberis aristata*	4.064±0.12	NMT 14
2	*Terminalia chebula*	2.31±0.02	NMT 5
3	*Emblica officinalis*	5.36±2.23	NMT 7
4	*Terminalia belerica*	5.16±0.04	NMT 7
5	*Cyperus rotundus*	6.18±0.01	NMT 8

The value are expressed as mean ± SD, (n=3); NMT-Not more than

ACID INSOLUBLE ASH

The total ash was used the acid insoluble ash content of individual raw materials was determined and results are enumerated in table.

Table: 12 Acid insoluble ash

S.NO	PLANT NAME	ACIN INSOLUBLE ASH(% w/w)	ACCEPTABLE LIMITS(w/w %)
1	*Berberis aristata*	0.34±0.08	NMT5
2	*Terminalia chebula*	0.45±0.02	NMT5
3	*Emblica officinalis*	1.17±0.16	NMT2
4	*Terminalia belerica*	0.68±0.01	NMT 1
5	*Cyperus rotundus*	2.27±0.04	NMT 4

The value are expressed as mean ± SD, (n=3); NMT-Not more than

WATER SOLUBLE ASH

The total ash content, the water soluble ash content of individual raw materials was determined and results are enumerated in table.

Table: 13 water soluble ash

S.NO	PLANT NAME	WATER SOLUBLE ASH(% w/w)	ACCEPTABLE LIMITS(w/w %)
1	*Berberis aristata*	0.78±0.01	NMT 3
2	*Terminalia chebula*	2.31±0.02	NMT 5
3	*Emblica officinalis*	1.76±0.37	NMT 3
4	*Terminalia belerica*	2.74±0.59	NMT 6
5	*Cyperus rotundus*	5.73±0.21	NMT 7

The value are expressed as mean ± SD, (n=3); NMT-Not more than

SULPHATED ASH

The total ash content, the Sulphated ash ash content of individual raw materials was determined and results are enumerated in table.

Table: 14 Sulphated ash

S.NO	PLANT NAME	SULPHATED ASH(% w/w)
1	*Berberis aristata*	1.25±0.02
2	*Terminalia chebula*	0.12±0.02
3	*Emblica officinalis*	1.5±0.12
4	*Terminalia belerica*	2.2±0.04
5	*Cyperus rotundus*	2.8 ±0.12

The value are expressed as mean ± SD, (n=3)

DETERMINATION OF EXTRACTIVE VALUES

Water Soluble Extractive Value

Water soluble Extractive values for the raw materials were determined and the results are tabulated in table.

Table: 15 Water soluble Extractive

S.NO	PLANT NAME	WATER SOLUBLE EXTRACTIVE (%w/w)	ACCEPTABLE LIMITS(w/w %)
1	*Berberis aristata*	14.29±0.04	NLT 8
2	*Terminalia chebula*	61.94±0.17	NLT 60
3	*Emblica officinalis*	51.63±0.20	NLT 50
4	*Terminalia belerica*	2.61±0.69	NLT 35
5	*Cyperus rotundus*	12.54±0.15	NLT 11

The value are expressed as mean ± SD, (n=3); NMT-Not more than

ALCOHOL SOLUBLE EXTRACTIVE VALUE

Alcohol soluble Extractive values for the raw materials were determined (90% ethanol) and the results are enumerated in table.

Table: 16 Alcohol soluble extractive

S.NO	PLANT NAME	ALCOHOL SOLUBLE EXTRACTIVE (%w/w)	ACCEPTABLE LIMITS(w/w %)
1	*Berberis aristata*	9.82±0.17	NLT 6
2	*Terminalia chebula*	42.66±0.16	NLT 40
3	*Emblica officinalis*	42.36±0.22	NLT 40
4	*Terminalia belerica*	14.88±0.28	NLT 8
5	*Cyperus rotundus*	8.08±20	NLT 5

The value are expressed as mean ± SD, (n=3); NMT-Not more than

ETHER SOLUBLE EXTRACTIVE VALUE

Ether soluble extractive values for the raw materials were determined and the results are enumerated in table.

Table : 17 Ether soluble extractive value

S. NO	PLANT NAME	ETHER SOLUBLE EXTRACTIVE(%w/w)
1.	*Berberis aristata*	9.28±0.64
2.	*Terminalia chebula*	8.35±0.42
3.	*Emblica officinalis*	12.68±0.24
4	*Terminalia belerica*	8.50±0.25
5	*Cyperus rotundus*	9.25±0.24

The value are expressed as mean ± SD, (n=3)

ANALYSIS OF HEAVY METAL

Estimation of heavy metals in the raw materials were carried out and the results were recorded and detailed in table.

Table: 18 Test for heavy metals

S.NO	PLANT NAME	OBSERVATION (in ppm)			
		Arsenic (NMT 5)	Lead (NMT 10)	Cadmium (NMT 0.3)	Mercury (NMT 0.5)
1	*Berberis aristata*	0.002	0.001	0.024	0.004
2	*Terminalia chebula*	0.004	0.003	0.005	0.002
3	*Emblica officinalis*	0.002	0.004	0.001	0.001
4	*Terminalia belerica*	0.001	0.005	0.04	0.04
5	*Cyperus rotundus*	0.005	0.002	0.002	0.004

The estimation of heavy metals in the sample revealed heavy metals are within the prescribed limits. It is safe and does not cause any harm on consumption.

MICROBIAL LOAD ANALYSIS

Table: 19 Microbial load analysis

S. No.	Parameters	*Berberis aristata*	Terminalia chebula	Emblica officinalis	Terminalia belerica	Cyperus rotundus
1	Total aerobic count(NMT 1000cfu/g)	Nil	Nil	100	100	Nil
2	Yeast and mould count (NMT 100cfu/g)	Nil	Nil	Nil	Nil	Nil
3	*E.coli* (To be absent)	Absent	Absent	Absent	Absent	Absent
4	*Salmonella* (To be absent)	Absent	Absent	Absent	Absent	Absent
5	*Pseudomonas*(To be absent)	Absent	Absent	Absent	Absent	Absent
6	*Staphylocoucus*(To be absent)	Absent	Absent	Absent	Absent	Absent
7	*Shigella* (To be absent)	Absent	Absent	Absent	Absent	Absent

From the results, it is shown that the powdered raw materials complies with the WHO standards for Microbial load analysis and hence it is safer to be taken internally.

PREPARATION OF EXTRACTS

The shade dried crude dried drugs of *Berberis aristata (*dried Stem), *Terminalia chebula* (pericarp of matured fruit), *Emblica officinalis* (pericarp of dried matured fruit),*Terminalia belerica* (pericarp of dried ripe fruit) and *Cyperus rotundus* (dried rhizome) weres extracted in soxhlet extractor with ethanol . All the extracts were concentrated using rotary vacuum evaporator. The percentage yield was calculated for every extract in terms of dried weight of plant material. The colour and consistency of the concentrated extracts are given in table.

Table. 20 Percentage yield of various extracts

S. no	Plant name	Method of extraction	Physical nature	Colour	Yield %w/w
1	*Berberis aristata*	Continuous hot percolation	Semi solid	Dark brown	**5.29**
2	*Terminalia chebula*				**22.05**
3	*Emblica officinalis*				**16.9**
4	*Terminalia belerica*				**16.16**
5	*Cyperus rotundus*				**8.75**

PHYTOCHEMICAL ANALYSIS

The chemical tests for various Phytoconstituents in the raw materials were carried out and the results were recorded and detailed in table.

Table: 21 Phytochemical analysis

Phytoc-constituents	*Berberis aristata*	*Terminalia chebula*	*Emblica officinalis*	*Terminalia belerica*	*Cyperus rotundus*
Phenolic compounds	_	+	+	+	+
Flavanoids	+	+	+	+	+
Tannins	_	+	+	+	+
Alkaloids	+	+	+	_	+
Steroids	+	+	+	_	_
Glycosides	+	+	+	+	+
Saponins	+	+	_	+	+
Proteins	+	+	+	+	+
Carbohydrates	+	+	+	+	+
Terpenoids	+	+	_	+	_

FLUORESCENCE ANALYSIS

Table 22: Fluorescence analysis of raw materials

SAMPLE	BEFORE TREATMENT			AFTER TREATING WITH 50 %HCL			AFTER TREATING WITH 50% NAOH		
	Ordinary Light	Short UV	Long UV	Ordinary Light	Short UV	Long UV	Ordinary light	Short UV	Long UV
Berberis aristata	Light yellow	Black	Black	Brown	Brown	Brown	Black	Black	Dark brown
Terminalia chebula	Light yellow	Brown	Dark brown	Light brown	Brownish Green	Dark brown	Light brown	Greenish brown	Dark brown
Emblica officinalis	Dark brown	Light brown	Black	Brown	Black	Dark brown	Light brown	Black	Black
Terminalia belerica	Dark brown	Brown	Brown	Brown	Brownish Black	Dark brown	Light brown	Brown	Dark brown
Cyperus rotundus	Black	Black	Light brown	Brown	Brown	Dark brown	Light brown	Dull brown	Light brown

CHROMATOGRAPHIC ANALYSIS

The chromatographic analysis for each individual ethanolic extract and polyherbal formulation was performed and the results were given in the table.

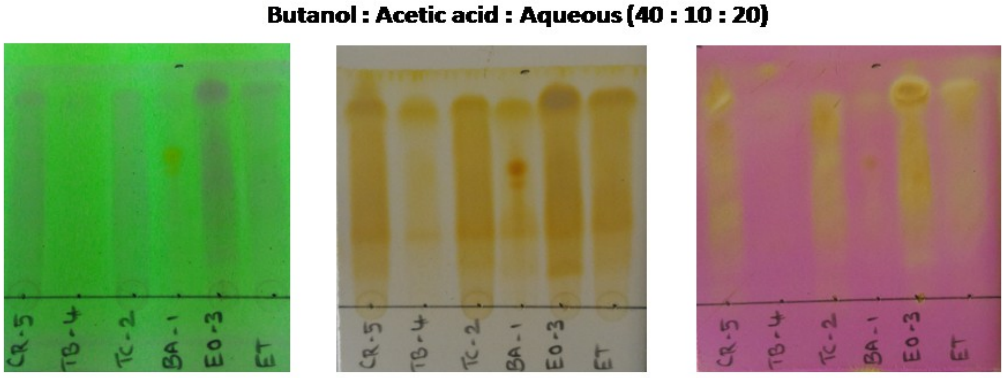


Figure:Thin layer chromatograpy of individual extract and PHF

CR- ***Cyoerus rotundus,*** **TB-** ***Terminalia belerica,*** **TC-** ***Terminalia chebula,***

BA - ***Berberis aristata,*** **EO -** ***Emblica officinalis,*** **ET - Poly herbal formulation**

PHF - POLYHERBAL FORMULATION

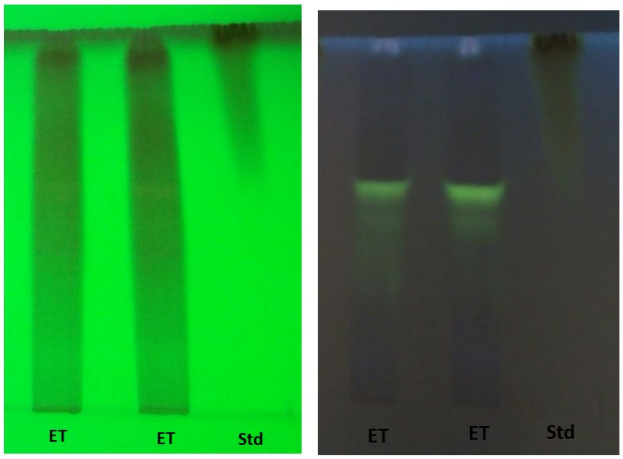
Table 23: Thin Layer Chromatography Rf values of the extracts

Extract	Solvent system	Number of spots	Distance travelled by the solute (cm)	Distance travelled by the solvent (cm)	Rf value (cm)
Cyperus rotundus	Butanol: Acetic acid: Aqueous (40: 10: 20)	4	2.8; 2.5; 1.4; 0.5	3	0.93; 0.83; 0.46; 0.16
Terminalia belerica		3	2.5; 1.9; 0.9	3	0.83; 0.63; 0.3
Terminalia chebula		4	2.6; 2.2; 0.9; 0.6	3	0.86; 0.73; 0.3; 0.2
Berberis aristata		6	2.6; 1.8; 1.5; 1.2; 1.0; 0.5	3	0.86; 0.6; 0.5; 0.4; 0.33; 0.16
Emblica officinalis		6	2.6; 2.5; 2.0; 1.5; 1.0; 0.5	3	0.86; 0.83; 0.66; 0.5; 0.33; 0.16
ET (Polyherbal formulation)		6	2.6; 2.4; 2.2; 1.4; 1.0; 0.5	3	0.86; 0.8; 0.73; 0.46; 0.33; 0.16

HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY

HPTLC was performed for the ethanolic extraction of polyherbal formulation**.** The chromatographic conditions were carried was detailed in materials of this study. There were six peaks observed with different R_f values.

Mobile Phase : Butanol-Acetic Acid-Water (4:1:5).



ET- polyherbal extract powder, Std -Flavanoid

HPTLC FINGER PRINTING OF THE POLYHERBAL EXTRACT

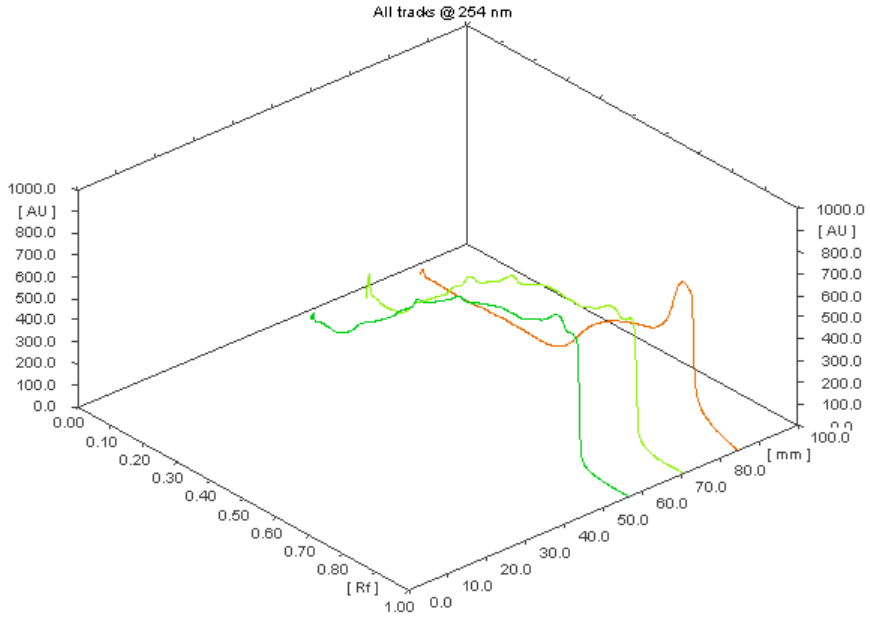


Fig23: HPTLC of finger print data

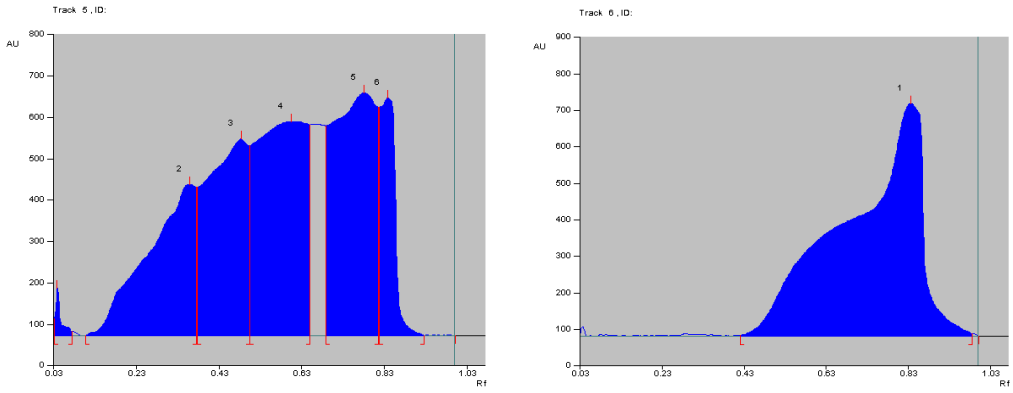


Table 24: HPTLC finger printing spectral values

Peak	Rf value	Assigned substance
1	0.05	Unknown
2	0.31	Unknown
3	0.48	Unknown
4	0.59	Unknown
5	0.78	Unknown
6	0.97	**Standard**

The HPTLC finger printing of the polyherbal ethanolic extracts showed the above tabulated R_f values with corresponding peak area.

HPTLC fingerprint is one of the versatile tools for qualitative and quantitative analysis of active constituents of multicomponent sample and also a diagnostic method to find out the adulterants to check purity.

PREFORMULATION STUDIES

Totally four trials of formulation were carried out using different choices of excipients considering different facts of manufacturing problems as well as quality defects in mind. All the resultant formulations were evaluated for their flow property, uniformity of filling, uniformity of weight, moisture content and disintegration time.

Table 25 :Evaluation of trial batches

Parameters	Trial 1	Trial 2	Trial 3	Trial 4
Bulk density ($g/cm^{2)}$	0.50±0.04	0.60±0.05	0.55±0.01	0.54±0.04
Tapped density($g/cm^{2)}$	0.61±0.02	0.53±0.03	0.54±0.03	0.57±0.04
Compressibility index (%w/w)	09.99±0.03	09.09±0.63	5.55±0.91	4.71±0.04
Hausner's Ratio	1.35±0.02	1.43±0.02	1.13±0.12	1.03±0.12
Angle of repose (degrees)	35.05±1.0	34.66±0.02	43.03±3.78	33.03±3.78

The value are expressed as mean ± SD, (n=5); NMT-Not more than

As per the standards, the flow property of the blend to be filled in the capsule should be in good range and was confirmed by the above parameters. Trail batch IV showed excellent flow characters and batch IV was taken for capsule filling.

Table 26: Evaluation Of Inprocess Parameters

PARAMETER	TRAIL I	TRAIL II	TRAIL III	TRAIL IV
Flow property	Poor flow	Poor flow	Fair	Good
Uniformity of Filling	-	-	Uniform	Uniform
Uniformity of Weight	-	-	Less weight	Uniform
Moisture content	-	-	Within the Limit	Within the limit
Disintegration time	-	-	Within the Limit	Within the limit

The trial IV flow properties were Excellent and all parameter were within the Specified limits. So, fourth trial was chosen for further studies

STANDARDISATION OF THE FINISHED PRODUCT

The final formulation was analyzed for its quality control parameters in three trials. The mean value was obtained and Standard deviation was calculated. Wherever there were no official standard, limits for each parameter was established based on trial and error analysis of Trial IV batch capsules.

EVALUATION OF CAPSULES

1. Description

"Light brown " coloured granules packed in "0" size blue capsules. The polyherbal capsules were evaluated for organoleptic characters which include colour, odour, taste and nature.

Table 27. Organoleptic Characters

S.NO	PARAMETER	OBSERVATION
1.	Description	Light brown granule in blue cap and body "0" size capsule
2.	Colour	Light brown granule
3.	Odour	Characteristic odour
4.	Taste	Bitter tate

Table 28. Physical Parameters

S.NO	PARAMETER	OBSERVATION
1.	pH (1% aqueous solution)	7.33 ± 0.21
2.	Moisture content	3.98 ± 0.5%w/w
3.	Uniformity of weight	519.3 ±3.4mg
4.	Disintegration time	2' 32secs ± 0.34

Results are reported as Mean ± Standard deviation.

- 1% aqueous solution of polyherbal formulation showed neutral pH.
- The average weight of the capsules was calculated as per I.P and the obtained value was within the limit (±7.5%).
- Disintegration time of the polyherbal capsule was performed as per IP and the obtained value showed that the capsule will be disintegrated with in the prescribed time for the absorption

Table 29. Physicochemical Parameters Of Capsule

S.NO	PARAMETERS	OBSERVATIONS (%w/w)
1.	Total ash	3.22 ± 0.33
2.	Acid insoluble ash	3.09± 0.46
3.	Sulphated ash	1.63 ± 0.24
4.	Water soluble extractive	5.4 ± 0.53
5.	Ethanol soluble extractive	1.16 ± 0.11
6.	Ether soluble extractive	1.21 ± 0.24

Results are reported as Mean ± Standard deviation.

PHYTOCHEMICAL ANALYSIS

PRELIMINARY PHYTOCHEMICAL SCREENING OF CAPSULE

Table 30: Preliminary phytochemical screening for polyherbal formualtion

S. No	Phytoc-constituents	Observation
1	Phenolic compounds	+
2	Flavanoids	+
3	Tannins	+
4	Alkaloids	+
5	Steroids	+
6	Glycosides	+
7	Saponins	+
8	Proteins	+
9	Carbohydrates	+
10	Terpenoids	+

QUANTITATIVE ESTIMATION OF PHYTOCONSTITUENT

The Polyherbal formulation was found to contain various phytochemical constituents and hence it is desirable to quantify few of them in order to establish a standard to maintain its quality. Among them the estimation of total phenolics, Flavanoids, and Tannin content in the ethanolic extract were decided to be taken as parameters. Samples were drawn from three random samples of polyherbal capsules and the total Phenolics, Flavonoids, and Tannin content present in them were estimated.

TOTAL PHENOLIC CONTENT

Table:31 Total Phenolic content

S.NO	Concentration of standard solution(µg)	ABSORBANCE (765nm)
1	2	0.016
2	4	0.028
3	6	0.038
4	8	0.052
5	10	0.063
6	PHF	0.058

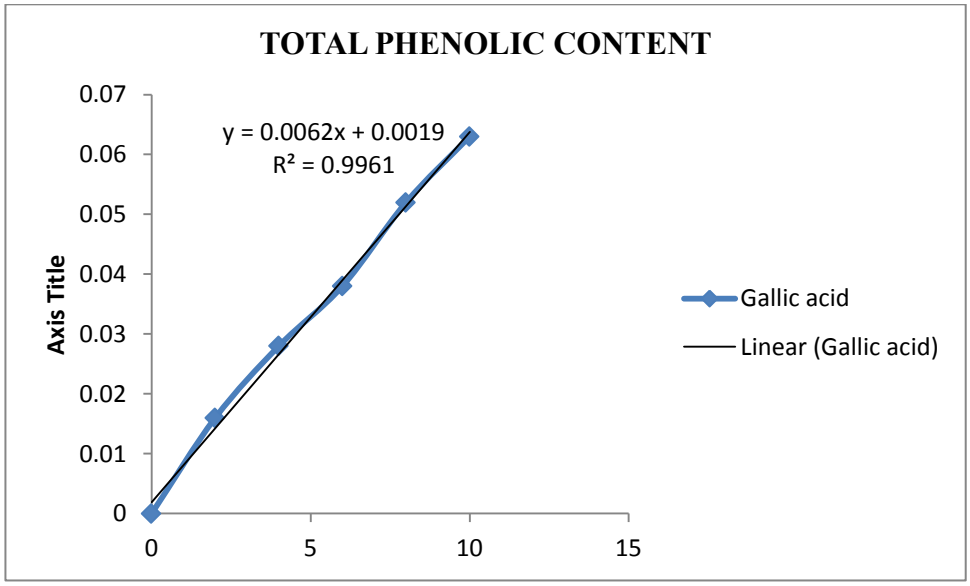


Fig 24: Calibration curve of Phenoilc conent

Comparison of standard (Gallic acid) absorbance at various concentration (2 µg to10 µg) and polyherbal formulation. Sample absorbance corresponds at standard absorbance at a concentration of 9µg. Hence the amount of flavanoid s present in formulation found to be 9µg.

TOTAL TANNIN CONTENT

Table:32 **Total tannin content**

S.NO	Concentration of solution(µg)	ABSORBANCE (510nm)
1	0	0
2	5	0.08
3	10	0.16
4	15	0.25
5	20	0.32
6	25	0.37
6	PHF	0.33

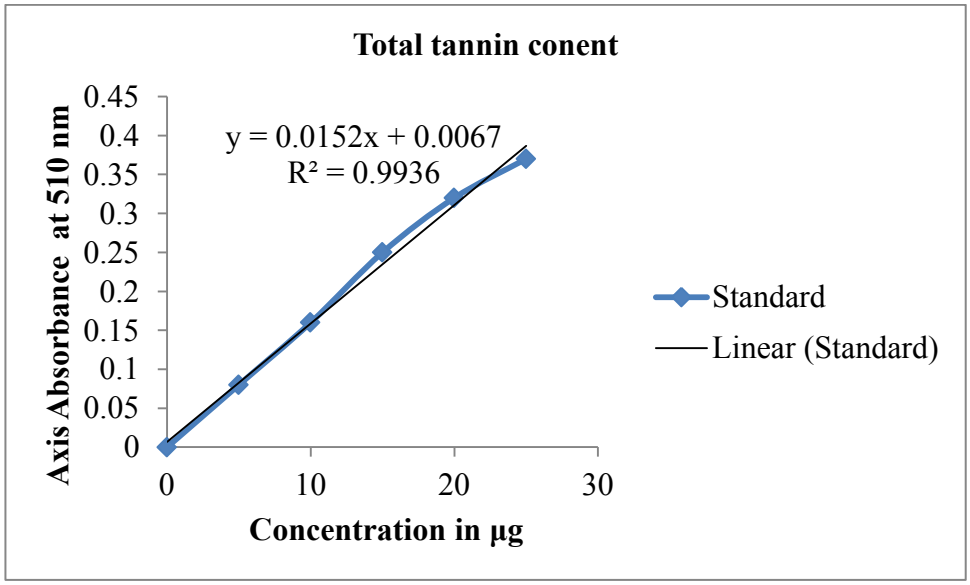


Fig 25: Calibration curve of total tannin conent

Comparison of standard absorbance at various concentration (5 µg to25 µg) and polyherbal formulation. Sample absorbance corresponds at standard absorbance at a concentration of 22µg. Hence the amount of tannin s present in formulation found to be 22µg

TOTAL FLAVONOID CONTENT

Table 33: **Total flavonoid conent**

S.NO	Concentration of standard solution(µg)	ABSORBANCE (765nm)
1	20	0.11
2	40	0.14
3	60	0.17
4	80	0.22
5	100	0.26
6	PHF	0.17

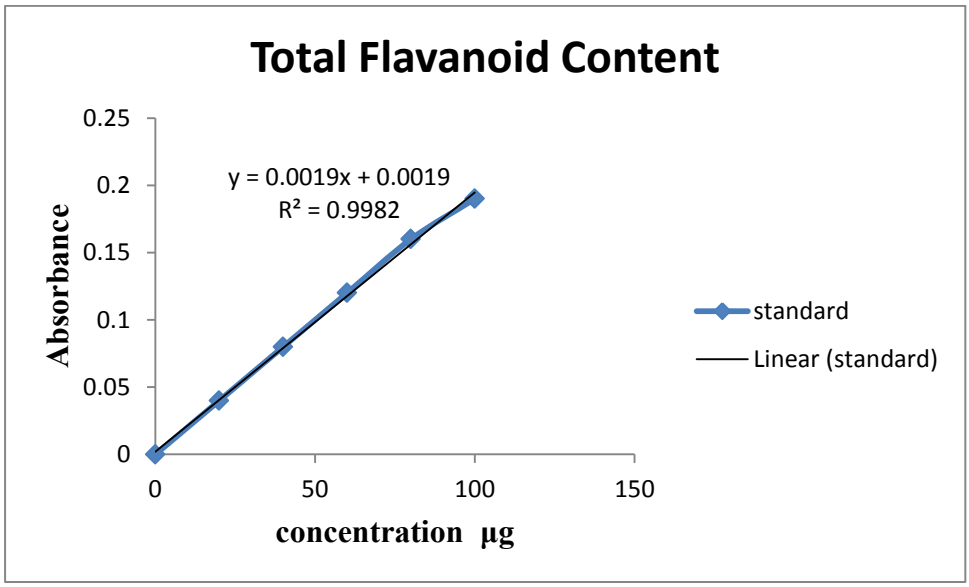


Fig 26: **Calibration curve of total flavanoid conent**

Comparison of standard (Quercertin) absorbance at various concentration (20 µg to100 µg) and polyherbal formulation. Sample absorbance corresponds at standard absorbance at a concentration of 63µg. Hence the amount of flavanoid s present in formulation found to be 63 µg

The estimated amounts of phenolics, Flavanoids, and Tannins were enumerated in the Table 34.

Table 34: Quantitative estimation of phytoconstituents

S.NO	PARAMETER	OBSERVATION (%w/w)
1	Total tannin content	0.54±0.15
2	Total flavonoid content	3.25±0.37
3	Total phenolic content	1.75±0.21

Result (n=3) are reported as Mean ± Standard deviation

From the results obtained it is determined that the average content of phenolics, Flavonoids and Tannins were present in the Polyherbal formulation.

HEAVY METALS ANALYSIS

Capsules were analyzed for the heavy metals which include Arsenic, Cadmium, Lead and Mercury. The results are as follows,

Table 35: Quantitative Heavy metals analysis

S.NO.	HEAVY METALS	OBSERVATION (in ppm)	LIMITS (in ppm)
1.	Arsenic	0.02	5
2.	Cadmium	0.02	0.3
3.	Lead	0.06	10
5.	Mercury	0.01	0.5

From the results it is shown that the Polyherbal formulation complies with the heavy metals limits of the WHO guidelines and hence it is safe to be taken internally.

MICROBIAL LOAD ANALYSIS:

Microbial screening is done for the Polyherbal formulation results obtained were detailed in table.

Table 36: Microbial load analysis

S.NO	PARAMETER	RESULT	Limits as per WHO
1	Total aerobic count	196cfu/g	NMT 1000 cfu/g
2	Yeast and Mould	15cfu/g	NMT 100 cfu/g
3	*E.Coli*	Absent	Should be absent
4	*Salmonella*	Absent	Should be absent
5	*Pseudomonas*	Absent	Should be absent
6	*Streptococcus*	Absent	Should be absent
7	*Shigella*	Absent	Should be absent

(Note: *cfu – Colony forming units*)

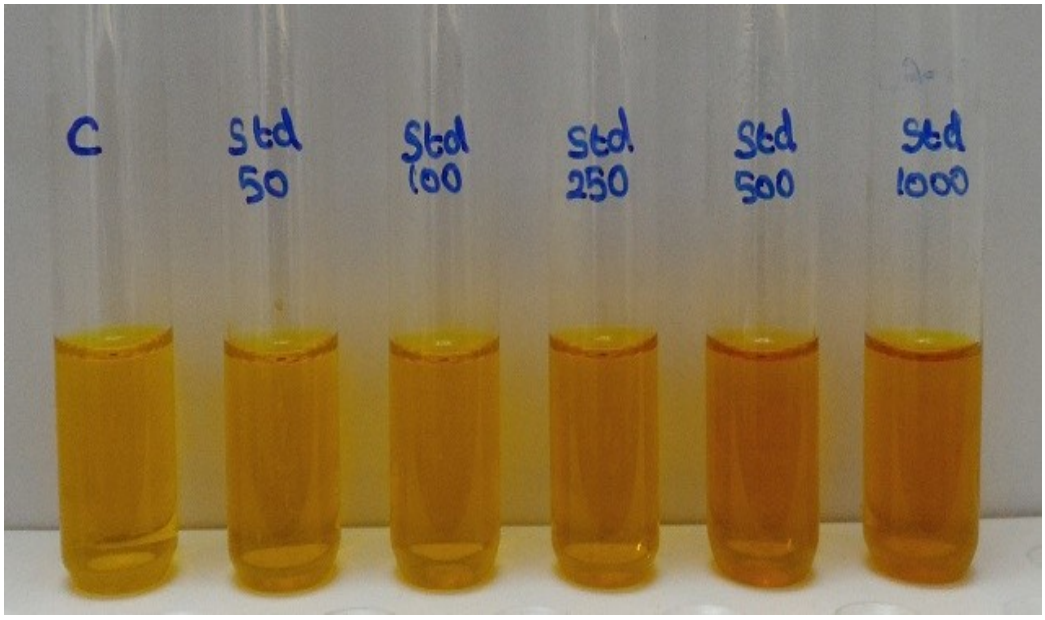
From the results, it is shown that the formulation complies with the WHO standards for Microbial load analysis and hence it is safer to be taken internally.

PHARMACOLOGICAL STUDIES

INVITRO ANTI-DIABETIC ACTIVITY

α -Amylase Inhibition Assay

STANDARD ACARBOSE



POLYHERBAL FORMULATION

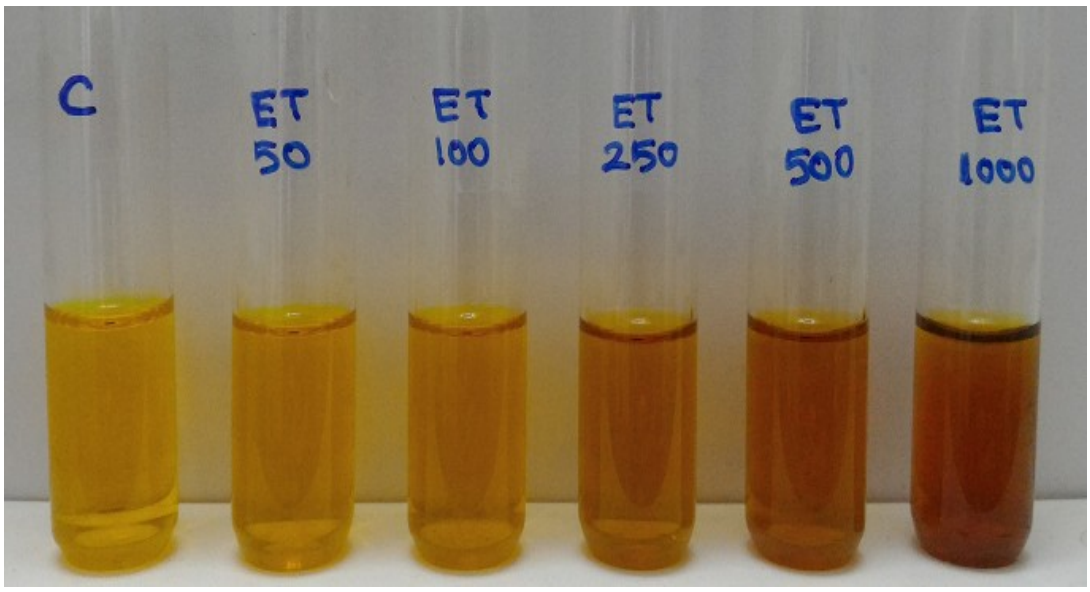
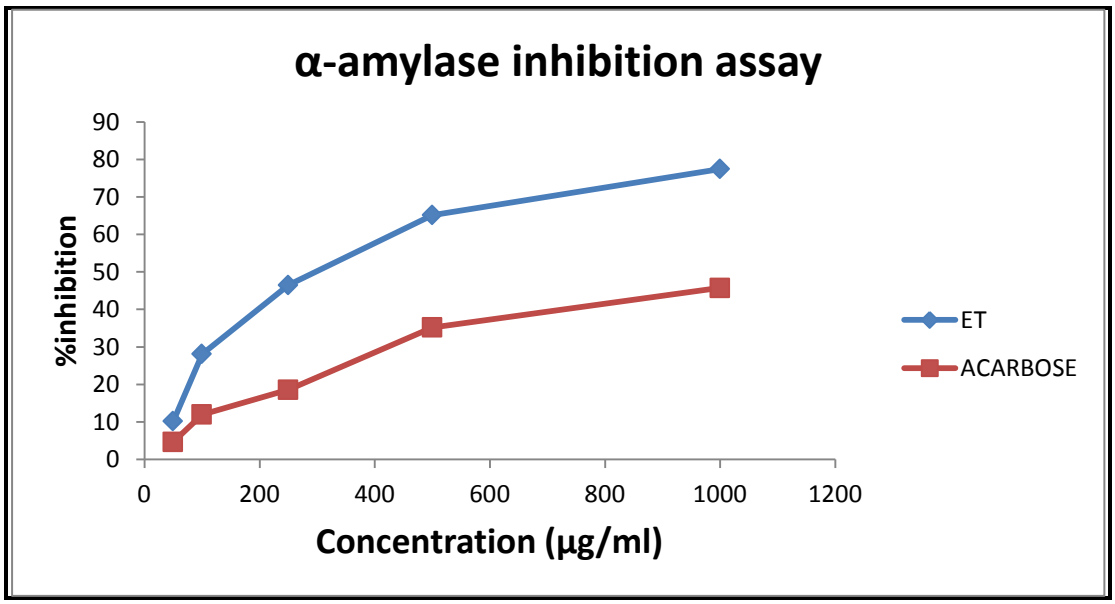


Table.37. I*n vitro* anti – diabetic activity

Compounds	Concentration	Absorbance	S-C	S-C/S	% inhibition	IC50
ACARBOSE	50µg	0.193	0.009	0.04663212	4.663212435	
	100µg	0.209	0.025	0.11961722	11.96172249	
	250µg	0.226	0.042	0.18584071	18.5840708	**1600.79**
	500µg	0.284	0.1	0.35211268	35.21126761	
	1000µg	0.339	0.155	0.45722714	45.72271386	
	Control	**0.184**				
PHF	50µg	0.205	0.021	0.10243902	10.24390244	
	100µg	0.256	0.072	0.28125	28.125	
	250µg	0.344	0.16	0.46511628	46.51162791	**279.21**
	500µg	0.528	0.344	0.65151515	65.15151515	
	1000µg	0.816	0.632	0.7745098	77.45098039	
	Control	**0.184**				

Fig 27: Graphical representation of the α-amylase inhibition assay



ET- POLYHERBAL FORMULATION

IN VIVO ANTI-DIABETIC ACTIVITY

ACUTE TOXICITY STUDY

Behavioural and physical observation of polyherbal formulation treated rats (2000mg/kg body weight)

Table : 38 Observation of acute toxicity study

OBSERVATION	30 mins	4 hrs	14 hrs	24 hrs
Body weight	No change	No change	No change	No change
Preterminal deaths	Absent	Absent	Absent	Absent
Cage side observation	Normal	Normal	Normal	Normal
Motor activity	Normal	Normal	Normal	Normal
Convulsions	Absent	Absent	Absent	Absent
Piloerection	Absent	Absent	Absent	Absent
Lacrimation	Normal	Normal	Normal	Normal
Salivation	Normal	Normal	Normal	Normal
Respiration	Normal	Normal	Normal	Normal
Skin color	Normal	Normal	Normal	Normal
Diarrhoea	Absent	Absent	Absent	Absent
Loss of corneal reflex	Normal	Normal	Normal	Normal
Loss of pinnal reflex	Normal	Normal	Normal	Normal
Grooming	Absent	Absent	Absent	Absent
Sedation	Normal	Normal	Normal	Normal
Excitation	Normal	Normal	Normal	Normal
Aggression	Normal	Normal	Normal	Normal

The results of acute toxicity study are shown in table .There were no morbidity and mortality observed for polyherbal formulation treated animals upto 2000 mg/kg.

INVIVO ANTIDIABETIC ACTIVITY

ANTIDIABETIC EFFECT OF HERBAL FORMULATION IN STREPTOZOTOCIN AND NICOTIMANIDE INDUCED DIABETIC RATS

The Albino Wistar rats were divided into four different Groups of six animals each as follows.

Group I : Diabetic rats treated with normal saline

Group II : Diabetic rats treated with PHF (200 mg/kg)

Group III : Diabetic rats treated with PHF (400 mg/kg)

Group IV : Diabetic rats treated with Glibenclamide (0.25 mg/kg).

Table: 39 The effect of polyherbal formulation on fasting blood glucose levels (mg/dl) in STZ and NIC induced diabetic rats

Treatment	Blood glucose level in mg/dl					
	Before induction	After induction (Initial day)	7th day	14th day	21st day	28th day
Diabetic control	78.5±1.60	215.33±2.71	270.11±2.24	302.19±0.73	326.92±1.20	346±1.60
PHF 200 mg /mg/kg	76.16±1.51	217±2.71***	200.6±3.38***	115.5±1.69***	99.21±1.52***	89.21±1.52***
PHF 400 mg/kg	76.66±0.84	218.33±7.03***	183.2±2.06***	112.21±1.9***	86.16±1.27***	78.51±0.83***
Glibenclamide 0.25mg/kg	74.5±1.58	220±3.65***	180.73±2.87***	100.7±2.01***	82.83±0.83***	74.21±0.84***

PHF: Polyherbal formulation. Values are expressed as mean±SEM (n=6). ***P<0.001 compared to diabetic control (one way ANOVA followed by Dunnett's t test) STZ: Streptozotocin; NIC: Nicotimanide

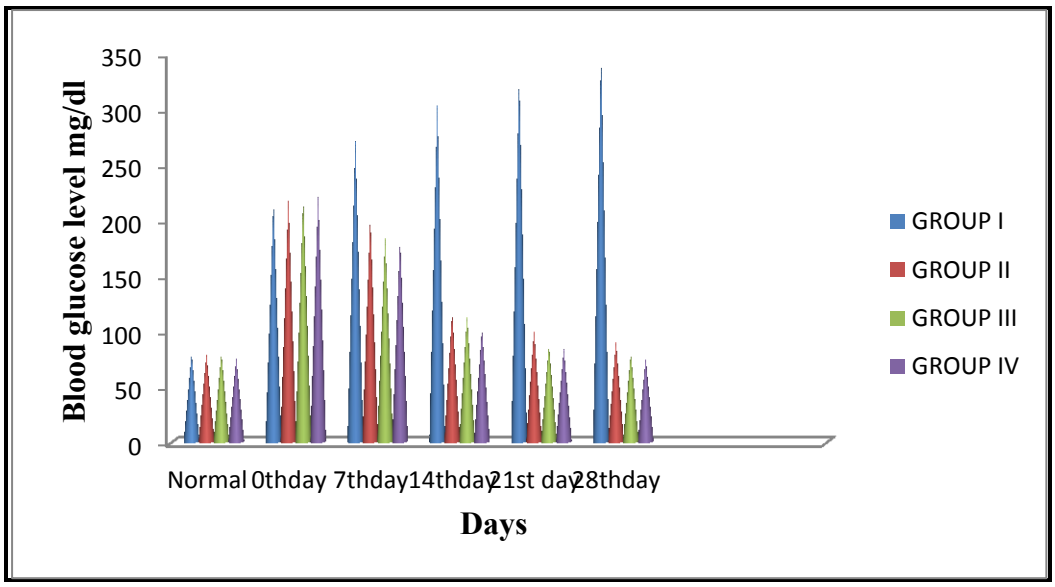


Fig 28: Graphical representation for fasting blood glucose level in *albino rats*

Table 40: EFFECT OF POLYHERBAL FORMULATION ON PLASMA LIPID PROFILE IN EXPERIMENTAL RATS IN EACH GROUP

GROUP	Lipid profile (mg/dl)				
	Triglyceride	Total cholesterol	HDL	LDL	VLDL
Before induction of diabetes	62.13±0.32	72.14±1.89	31.26±1.80	37.32±1.26	13.20±0.17
Diabetic control	161.04±0.63	142.01±0.62	13.23±0.20	92.62±0.17	41.22±0.27
PHF 200 mg/kg	65.10±0.46***	80.11±0.52***	27.64±0.95***	42.10±0.08***	15.12±0.77***
PHF 400 mg/kg	67.12±1.02***	78.20±1.06***	29.10±0.84***	39.18±0.94***	14.65±0.47***
Glienclamide (0.25 mg/kg)	68.05±0.10***	77.11±0.44***	30.47±0.37***	39.97±1.09***	14.00±0.18***

PHF: Polyherbal formulation. Values are expressed as the mean±SEM (n=6). *P<0.05, **P<0.01, ***P<0.001 compared to diabetic control animals (one way ANOVA followed by a Dunnett's t-test) HDL: High density lipoprotein; LDL: Low density lipoprotein; VLDL: Very low density lipoprotein.

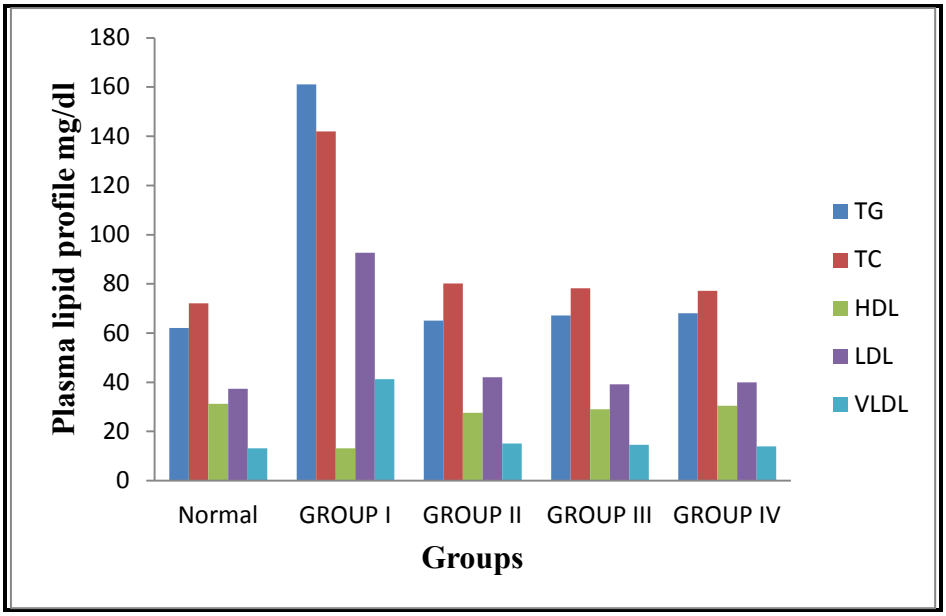


Fig 29: Graphical representation of plasma lipid profile in *albino rats*

HDL: High density lipoprotein; LDL: Low density lipoprotein; VLDL: Very low density lipoprotein.

8. SUMMARY AND CONCLUSION

Herbal medicines are the oldest form of health care known to mankind. A number of traditional herbal medicinal practices have been adopted for the diagnostic prevention and treatment of various diseases.

Based on the extensive review of literature, five raw materials were selected for the formulated as polyherbal capsules and the antidiabetic potency was evaluated in animal model.

The herbal raw materials were analyzed for identity, quality and purity as per the standards prescribed by WHO and Ayurvedic Pharmacopeia of India.

The Physiochemical parameters like Loss on drying, ash values and extractive values were determined, which will help in preventing variation in quality of the drugs. Preliminary phytochemical investigation revealed the presence of various phytoconstituents such as alkaloid, steroids, glycosides, Flavonoids, Phenols, Tannins, and terpenoid in the raw materials.

The safety of the raw materials was analysed by heavy metals and microbial screening and the results found within the standard limits given by WHO.

The coarse powders of the selected plants were extracted by using ethanol as solvent. The ethanolic extracts were dried by freeze drying and used for the formulation.

TLC for the each individual plant extracts and polyherbal extract was performed. Further HPTLC fingerprinting of the polyherbal formulation was also performed and the resultant chromatogram showed the presence of peaks indicating different constituents. The chromatogram can be used as an index for the qualitative analysis of the formulation.

The dried polyherbal extract was optimized for its quality measures and its batch consistency by making four different trial batches (Trial I, II, III, IV).

The trials were subjected to preformulation parameters to confirm the uniformity and quality. The result concludes that the trial IV was excellent in all parameters and the values were found within the standard limits and it was used for formulate Polyherbal Capsule.

The developed polyherbal capsules were standardized for its Description, uniformity of weight, disintegration time, moisture content, pH, Physiochemical parameters, and phytochemical studies.

Quantitative estimation of phytoconstituents was done for flavonoid, phenols, and tannins.

The heavy metal analysis and the microbial load was carried out in polyherbal formulation as per the WHO Guidelines and found within the limits.

In vitro anti-diabetic activity was done by using α-amylase inhibition assay method. It possesses significant antidiabetic activity as compared to standard Acarbose.

Acute toxicity study was carried as per OECD guidelines 423 and the polyherbal capsules were found to be safe upto 2000 mg/Kg body weight. The developed and standardized polyherbal capsule was evaluated for its therapeutic efficacy by *in vivo* method using Streptozotocin induced diabetes.

Fasting blood glucose parameters and lipid profile were analyzed. The formulation showed significant effect compared to the normal range before induction of diabetes. The polyherformulation at the dose level of 400 mg/kg showed significant decrease in blood glucose level on 7^{th} day.

The elevated levels of total cholesterol, LDL and triglycerides were also reduced and increases HDL level. The phytochemical study showed the presence of flavonoids. This may be responsible for the potent anti diabetic activity.

Further studies are recommended for stability studies in the formulated polyherbal capsules and also clinical trials have to perform in future in Human Volunteers.

REFERENCES

1. Fact sheet no.134. World Health Organization. 2008-12-01. Retrieved 2009-05-02.
2. Sheetal Verma and S.P.Singh, Current and future status of herbal medicines,*Veterinary World* 2013; 1(11): 347-50.
3. Zeeshan Hasan.S, Vedant Misra, Swati Singh, Garvita Arora, Sunita Sharma and Sarika Sharma, Current status of herbal drugs and their future perspectives, Biological Forum – An International Journal 2009; 1(1),12 -17
4. Balandrin M.F, Klocke J.A, Wrtele E.S and Boilinger W.H. Content and purity of extract solasodine in some available species of solanum,Science and culture 1985; 56(5),214-216.
5. CraggG.M, NewmanD.J, Sander K.M,Natural products in drug discovery and development.*Journal of Natural Products* 1997 (60): 52-60.
6. Jone BE. Pharmaceutical capsules. 2nd ed. Edited by podczeck F and John BE, Pharmaceutical press 2004; 91, 242,449.
7. Neeraj Choudhary, Bhupinder Singh Sekhon. An overview of advances in the standardization of herbal drugs. J Pharm Educ Res.2011; 2(2) :55-70.
8. http: // www.pharmainfo.net/ Herbal medicines and its standardization 2007; 5(6).
9. Bhutani KK. Herbal medicines an Enigma and Challenge to Science and directions for new initiatives. Indian Journal of Natural Products. 2003; 19(1): .3-8.
10. [WHO] Fact sheet No.138
11. Irfan ali khan, Atiya Khanum. Herbal therapy for Diabetes, 1st edition. Ukaaz Publications. 2007; 87: 119-20.
12. Dean L, McEntyre J. The Genetic Landscape of Diabetes, Chapter 1, Introduction to Diabetes ,National Center for Biotechnology Information (US) 2004.
13. Harsh mohan, Textbook of pathology, 5th edition , Medical Publisher's Ltd, New Delhi 2005; 818-828.
14. Kumar CH, Dr. Kumar JNS, Ishaq BM, Rani GU, Prkash KV. Antidiabetic activity of a polyherbal preparation, Pharmacologyonline 2010; 2: 780-787.

15. Kaul S, Dewivedi Indigenous ayurvedic knowledge of some species in the treatment of human disease and disorders. International Journal of Pharma Life Sciences 2010: 1 : 44–49
16. Kumar T, Chandrashekhar KS ,Bauhinia purpurea Linn: a review of its Ethanobotany, phytochemical and pharmacological profile. Research Jounal of Medicinal Plant 5:420–431
17. Sharma R.K., Bhakawan dash, Caraka Samhita, Published by Chowkhamba Sanskrit Series Office, Varanasi, India Vol III; 306.
18. Gupta A, Jaiswal M, Prajapati PK, Shelf life of Ayurvedic dosage forms- Tradional view, current status and prospective need, Indian Journal of Traditional knowledge,2011;10,672-677.
19. Muhummaed Rizwan, Alangir khan ,Hira Nasir, Aslam javed,Syed Zawar Shah, Phytochemical and Biological Screening of *Berberis aristata*, Advancements in life Sciences 2017; 5(1): 1-07.
20. Hamsalakshmi, Suresh, Saravanababu,Silpa M, A review on Pharmacognostical, Phytochemical and anti hyperlipidemic potentials of *Terminalia Belerica*, World Journal of Pharmaceutical and Life Scinces 2017; 16,(10): 422-433.
21. Srishti Dhyani, Singh.DC , Suresh Chaubey, A Comparative Pharmacognostical Study of Rhizome of Mustaka and Nagar Mustaka Intretnational J.Res.Ayurveda Pharma 2016; 7(3): 3-58.
22. Sarvesh kumar Bharathi, Abishek kumar, Bhuwal Ram and Anil kumar singh, Pharmacognostical and Phytochemical Evaluation of *Daruharidra*, World Journal of Pharmaceutical and Life Scinces 2016; 2(4): 419-430.
23. Nishant Pathak, Gyanendra Kumar and Rakesh Chandra Chaurasia. Evaluation of anti-diabetic activity of commercially available extracts of *Phyllanthus emblica* in Streptozocin induced diabetic rats. Int J Pharm Bio Sci 2016 Oct; 7(4): 139 - 145.

24. Alagar raja M, Shailaja V, David Banji, Rao K.N.V, SelvaKumar.D, Evaluation of Standardiasation parameters, Pharamacogonstic Study, Preliminary Phytochemical Screening and *In vitro* Ant diabetic ability of *Emblica officinalis* fruits as per WHO guideline, Journal of Pharmacognostical and Phytochemical 2014; 3(4): 21-28.

25. Aji Abraham, Lizzy Mathew and Sarala Samuel, Pharmacognostical Studies of the fruits of *Terminalia Belerica* (Gaertn.) Roscb, journal of Pharmacognosy and phytochemistry 2014; 3(2): 45-52.

26. Nidhi Khemaka, Harisha C R, Shukla V J, Galib R, Patgiri, Prajapati P K, Preliminary Pharmacognostical and Phytochemical Evaluation of Prashata and Aprashsata Haritaki (*Terminalia Chebula* Retz), International Journal of Ayurvedic medicine 2014; 5(3): 257-268.

27. Sri Rajani Sivapalan, Prince Jeyadevan Physiochemical and Phyto-chemical studies of Rhizome of *cyperus rotundus* Linn, International journal of pharmacology and pharmaceutical Technology 2012,1(2): 42-46.

28. Disha Arora, Ashish Sutee,Phytochemical and Microscopical investigation on *Emblica officinalis*.Gaertn, International Journal of Pharamacognostical and Phytochemical research 2012; 4(1): 1-4.

29. Surendra Kumar Sharma and Ajay pal singh, Morphological, Microscopical and Phytochemical investigation on the rhizomes of *cyperus rotundus* Linn, Research journal of Pharmaceutical Biological and Chemical Science July-September 2011; 2(3): 3: 798-806.

30. Nitinkumar Upwar, Roshan Patel, Naheed Waseem1, Naveen Kumar Mahobia, Hypoglycemic Effect Of Methanolic Extract of *Berberis Aristata* Dc Stem on normal and Streptozotocin Induced Diabetic Rats. International Journal of Pharmacy and Pharmaceutical Sciences 2011; 3(1): 222-224.

31. Sabu MC, Kuttan R, Antidiabetic and antioxidant activity of *Terminalia belerica.*Roxb. Indian Journal of Experimental Biology 2009; 47(4): 270-275

32. Gandhipuram periyasamy, Sentilkumar, Palanisamyarulselvan, Durairaj sathishkumar and Sorimuthu Pillar Subramanin. Antidiabetic effect of fruits of *Terminalia chebula* in Streptozotocin induced diabtic rats. Journal of Health Sciences 2006; 52(3): 283-291.

33. Nishikant A. Raut, Naresh J. Gaikwad. Antidiabetic activity of hydro-ethanolic extract of *Cyperus rotundus* in alloxan induced diabetes in rats. Fitoterapia 2006; 77(2): 585–588.

34. Dinesh jadhav, Medicinal plants of India, Scientific Publishers(India), Jodhpur 2008; Volume 2: 41-42.

35. Anonymous, The Ayurvedic Pharmacopoeia Of India, First edition, Ministry of health and family welfare, Govt. of india. The controller of publications, New Delhi, Part I, Vol. 2 :33-34.

36. Dinesh jadhav, Medicinal plants of India Scientific Publishers (India), Jodhpur 2008; Volume1: 228-229.

37. Anonymous. The Ayurvedic Pharmacopoeia Of India, ,First edition, Ministry of health and family welfare, Govt. of india, The controller of publications, New Delhi, part-I, Vol.3: 47-48.

38. Dinesh jadhav, Medicinal plants of India, Scientific Publishers(India), Jodhpur 2008; Volume 1: 188-189.

39. Anonymous. The Ayurvedic Pharmacopoeia Of India, First edition,Ministry of health and family welfare, Govt. of india, The controller of publiucations, New Delhi, part-I, Vol.1, 5-6.

40. Dinesh jadhav, Medicinal plants of India, Scientific Publishers(India), Jodhpur 2008; Volume 1: 227-228.

41. Dinesh jadhav, Medicinal plants of India, Scientific Publishers(India), Jodhpur 2008; Volume 1: 103-104.

42. Anonymous. The Ayurvedic Pharmacopoeia Of India,. Ministry of health and family welfare, Govt. of india, The controller of publiucations, New Delhi, Part I, Vol.4 : 129-130.
43. Anonymous. The Ayurvedic Pharmacopoeia of India, Vol.4. Ministry of Health and Family welfare, Govt. of India. The controller of publications, New Delhi. 2002; 158-165
44. Wallis TE. Analytical Microscopy- Its aims and methods in relation to foods, water, spices and drugs. Third Edition. Boston: Little, Brown and Company; 1965
45. Lira, Sergio, Peter Brush, Laurence Senak, Chi San Wu and Edward Malawer. The use of Inductively coupled plasma-optical emission spectroscopy in the determination of heavy meatls in crospovidone and povidone as a Replacement for the concomitant visual comparison test. Pharmacopoeial Forum. 2008: (3)4, 6-10.
46. WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues, World Health Organization, Geneva. 2007.
47. Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 2nd ed. London, Chapman and hall. 1973; 434.
48. Kokate CK, Purohit AP, Gokahle SB. Pharmacognosy. 24thed.Vallabh Prakashan, Pune. 2003; pp.108-109.
49. Gurdeep R, Chatwal, Sham K Anand, Instrumental methods of chemical analysis,Mumbai. Himalaya Publication House 2005; 2.272-2.302
50. Beckett AH, Stenlake JB, Practical Pharmaceutical chemistry 2nd Ed, New Delhi: CBS publishers, 2001,115-126.
51. Kasture A V, Mahadik K R, Wadokar S G, More H N. Chromatography, Pharmaceutical analysis. 15th edition. Pune. Nirali Publication. 2006.
52. Pharmaceutical preformulation by T.T Carlinsen published by Technomic Publishing Co.,p-1-6. 211-212.
53. Rowe RC, Shesky PJ, Quinn ME. *Hand book of Pharmaceutical Excipients.* 6th ed. London: pharmaceutical press and American pharmacists association; 2009

54. Jone BE. Pharmaceutical capsules 2nd ed. Edited by Podczeck F. Pharmaceutical press. 2004; 91, 242, 449.
55. Van Hostetler. Hard Capsules. In: Leon Lachman, Herbert AL, Joseph LK. The Theory and Practice of Industrial Pharmacy. 3rd ed. Varghese Publishing House, Bombay. 1991; 374.
56. Shah VK, Zalavadiya V, Sheth NR, Raval NK, Golwala DK. Standardization and assessment of preformulation parameters of rasayana Tablet. International Journal of Pharmaceutical Sciences and Drug Research. 2010;2(1): 58-62.
57. Remingtons Pharmaceutical Sciences, 17th ed. Mack Publishing Co.Easton, PA. 1985; pp.1633–1643.
58. Anonymous. Indian Pharmacopoeia, Vol.I, Govt of India, Ministry of Health and Family Welfare, Controller of Publication, Ghaziabad. 2007; A 53- 54.
59. Biju John, Sulaiman C T, Satheesh George and VRK Reddy, Total phenolics and flavonoids in Selected plants in Kerela, International Journal of Pharmacy and Pharmaceutical Sciences, 2014; 6(1); 406-408.
60. Barku VYA, Y Opoku-Boahen, E Owusu-Anash and EF Menash. Antioxidant activity and the estimation of total phenolic and flavonoid contents of the root extract of *Amaranthus spinosus.* Asian Journal of Plant Science and Research.2013; 3(1):69-74.
61. Shivakumar B S. Quantitative Determination of total content of phenol, flavonoid and tannin in leaf extract of *Barlaria buxifolia* Linn. Am. J. PharmTech Res. 2012
62. J. Agric. Food Chem. 2014, 62, 9507−9514
63. OECD Guideline for testing of chemicals, Acute Oral Toxicity – Acute Toxic Class Method, Original Guideline 423.March 1996; pp.1-14.
64. OECD. Guideline for testing of chemicals 423 (online); 2001 [cited on 2010 Feb 11]. Available from URL: http://iccvam. Niehs. Nih. Gov/SuppDocs/ OECD/OECD GL423. pdf.
65. Annadurai T, Muralidharan AR, Joseph T, Hsu MJ, Thomas PA, Geraldine P. Antihyperglycemic and antioxidant effects of a flavanone, naringenin, in

streptozotocin‐nicotinamide‐induced experimental diabetic rats. J Physiol Biochem 2012;68:307‐18.

66. Sharma U, Sahu RK, Roy A, Golwala DK. *In vivo* antidiabetic and antioxidant potential of *Stephania hernandifolia* in streptozotocin-induced-diabetic rats. Journal of Young Pharmacists. 2010; 2(3): 255-260.
67. Ratra M, Gupta R. Evaluation of antidiabetic activity of ethanol extracts of leaves and barks of *Alangium salvifolium* in streptozotocin-induced diabetic rats. UK Journal of Pharmaceutical and Biosciences. 2014; 3(3): 15-21.
68. Parasuraman S, Raveendran R, Kesavan R. Blood sample collection in small laboratory animals. J Pharmacol Pharmacother 2010;1:87‐93.
69. Samyal ML, Ahuja A, Ahmed Z. Evaluation of Antidiabetic Activity of Isolated Compound from *Ougeinia oojeinensis* Bark Extract in Diabetic Rats. UK Journal of Pharmaceutical and Biosciences. 2014; 2(5): 27-33.
70. Snehal S Patel, Rajendra S Shah and Ramesh K Goyal.Antihyerglcemic, Antihyerlipidemic ,antioxidant effects of Dihar a polyherbal formulation in Streptozotocin induced diabetic rats.,*Indian journal of Experimental biology*.200;47:564-570.
71. S.K.Gupta. Drug screening methods,2nd Ed,2009.p 614

सिद्ध केंद्रीय अनुसन्धान संस्थान

(सी.सी.आर.एस., चेन्नई, आयुष मंत्रालय, भारत सरकार)

अण्णा सरकारी अस्पताल परिसर, अरुम्बाक्कम, चेन्नई - 600106

SIDDHA CENTRAL RESEARCH INSTITUTE

(Central Council for Research in Siddha, Chennai,
Ministry of AYUSH, Government of India)
Anna Govt. Hospital Campus, Arumbakkam, Chennai - 600106
E-mail: crisiddha@gmail.com Phone: 044-26214925, 26214809

12.09.2017

AUTHENTICATION CERTIFICATE FOR 17091301-05

Certified that the drugs submitted by P. Vijayalakshmi, M.Pharm Second year, Dept. of Pharmacognosy, Madras Medical College, Chennai-03 are identified as:

SN	Botanical Name	Tamil Name	Part	Code
1.	*Berberis aristata* DC.	Maramanjal	Dried Stem	B17091301A
2.	*Terminalia chebula* Retz.	Katukkai	Pericarp	T17091302C
3.	*Terminalia bellerica* (Gaertn.)Roxb.	Tanrikkai	Pericarp	T17091303B
4.	*Emblica officinalis* Gaertn.	Nellikai	Pericarp	E17091304O
5.	*Cyperus rotundus* L.	Korai kizhangu	Rhizome	C17091305R

Continued in next page...

B17091301A

T17091302C

T17091303B

E17091304O

C17091305R

Dr. K.N. Sunil Kumar
R.O. and HOD Pharmacognosy

Dr. P. Sathiya Rajeswaran
Assistant Director (Siddha)
and In Charge

डॉ. पी. सत्तियराजेश्वरण/Dr. P. Sathiyarajeswaran
प्रभारी सहायक निदेशक (एस-II)/Assistant Director (S-II) I/C
सिद्ध केंद्रीय अनुसंधान संस्थान,
अण्णा सरकारी अस्पताल परिसर, अरुम्बाक्कम, चेन्नई-600 106
SIDDHA CENTRAL RESEARCH INSTITUTE
(Central Council for Research in [illegible] of AYUSH, Govt. of India)
Anna Govt. Hospital Campus, Arumbakkam, Chennai 600106

Madras Medical College, Chennai-600 003
Institutional Animal Ethics Committee
Proceedings
Present: Dr.Sudha Seshayyan, M.B.B.S, M.S (Anatomy)

Roc. No. 13/ AEL/IAEC/MMC,Date: 12.09.2017

Sub: MMC- IAEC - approval – regarding.

Ref: **IAEC** meeting held on 06.09.2017.

The following order is issued based on the approval of the Institutional Animal Ethics Committee held on 06.09.2017

Project ID.	13 /17.
CPCSEA registration number	1917 / ReBi/S/16/CPCSEA /25.10.2016
Name of the Researcher	Vijayalakshmi. P, M. Pharm II year, Department of Pharmacognosy
Name of the Guide	Dr.P.Muthusamy M.Pharm, Ph.D, B.L,
Title of the project	Development and standardization of polyherbal antidiabetic formulation
Date of submission of proposal to IAEC	03.08.2017
Date on which IAEC conducted	06.09.2017
Date of submission of modified proposal (if applicable)	06.09.2017
Date on which approved	06.09.2017
Validity of the approved proposal	1 year
Remarks: Wistar albino rats of either sex- 24 numbers approved.	

Chairperson
Institutional Animal Ethics Committee
Madras Medical College
Chennai -3

Dr. SUDHA SESHAYYAN
VICE PRINCIPAL
MADRAS MEDICAL COLLEGE
CHENNAI-600 003.

To
Dr.P.Muthusamy M.Pharm, Ph.D, B.L,
Assistant Professor, Dept. of Pharmacognosy,
College of Pharmacy,
MMC, Chennai -3.

Copy to
Special Veterinary Officer, Animal Experimental Laboratory
Madras Medical College, Chennai – 3.

DEPARTMENT OF PHARMACOGNOSY
SRI RAMACHANDRA COLLEGE OF PHARMACY
SRI RAMACHANDRA UNIVERSITY
(Declared under section 3 of the UGC Act, 1956)
Accredited by NAAC with 'A' Grade
Porur, Chennai - 600 116.

in association with
Society for Ethnopharmacology, Chennai Chapter

CERTIFICATE

This is to certify that Dr./~~Mr.~~/Ms. P. Vijayalakshmi .....

has participated as a Resource person / Delegate in the Pre conference workshop on ***"Recent Advances in Analytical Techniques - Drugs & Pharmaceuticals"*** *held on 27th June 2017, organized by Sri Ramachandra University, Porur, Chennai.*

This carries 2 Credits.

D. Chamundeeswari
Dr. D. CHAMUNDEESWARI
Principal &
Co-ordinator, SFE - Chennai Chapter

Dr. K.V. Somasundaram
Dr. K.V. SOMASUNDARAM
Dean of Faculties

"Skill and Will to Make and Serve Quality Pill"

69th IPC 2017

CHANDIGARH

22nd - 24th December, 2017

PHARMA VISION 2020

PHARMACISTS FOR A HEALTHY INDIA

Certificate

This is to certify that

Prof./Dr./Mr./Ms. P. Vijayalakshmi

has participated as Delegate / Volunteer

in the 69th Indian Pharmaceutical Congress

held at Chitkara University, Rajpura from December 22nd to 24th, 2017.

Dr. Mahesh Burande	Dr. Shailendra Saraf	Dr. Dhirender Kaushik	Dr. Ashish Baldi
President - IPCA	Chairman - LOC	Organizing Secretary	Chairman, Registration Committee - LOC

Organised by : Indian Pharmaceutical Congress Association (IPCA)

Hosted by : Association of Pharmaceutical Teachers of India (APTI)

COLLEGE OF PHARMACY

MADRAS MEDICAL COLLEGE

CHENNAI - 600 003.

Certificate

This is to certify that ~~Mr.~~ / Ms. VIJAYALAKSHMI . P ,

a student of II M.PHARM , *College of Pharmacy, Madras Medical College, Chennai has attended the* ***"Lecture series-2018"*** *conducted by the College of Pharmacy, Madras Medical College, Chennai - 03 on 11.01.2018.*

Dr. N. Jayshree, M.Pharm, Ph.D.,
Organizing Secretary,
Professor of Pharmacology,
Madras Medical College, Chennai.

Dr. A. Jerad Suresh, M.Pharm, Ph.D., M.B.A.,
Principal,
College of Pharmacy,
Madras Medical College, Chennai.

VELS
INSTITUTE OF SCIENCE, TECHNOLOGY & ADVANCED STUDIES (VISTAS)
(DEEMED TO BE UNIVERSITY Estd. u/s 3 OF THE UGC ACT, 1956)
NAAC ACCREDITED
PALLAVARAM - CHENNAI - INDIA

Sponsored by

AICTE
All India Council for Technical Education

Department of Pharmacognosy
SCHOOL OF PHARMACEUTICAL SCIENCES

AICTE
Sponsored

NATIONAL SEMINAR ON

CHALLENGES & OPPORTUNITIES IN GREEN NANOTECHNOLOGY

Certificate of Participation

This is to certify that Dr./Mr./Ms. P. VIJAYALAKSHMI has participated as a Resource person/ Presented e-poster/ Delegate/ Organizer in the National seminar on **"Challenges & Opportunities in Green Nanotechnology"** held on 25th January 2018, organized by the Department of Pharmacognosy, School of Pharmaceutical Sciences, VISTAS, Pallavaram, Chennai.

Dr. S. Jayakumari	Dr. P. Shanmugasundaram	Dr. P. Swaminathan
Organizing Secretary	Organizing Chairman	Vice Chancellor i/c

Mother Theresa Post Graduate and Research Institute of Health Sciences

(A Government of Puducherry Institution)

Department of Pharmacognosy,College of Pharmacy,Puducherry - 605 006
Accredited by NAAC with grade "A", Approved by UGC Under Section 2(f) & 12(B),PCI,AICTE & BOME
Permanently affiliated to Pondicherry University (A Central University).

7th National Conference on Recent Trends in Industrial Pharmacognosy - 2018

Theme : Pharmacognosy Vision 2030 - Paradigm Shift in Research Focus from Product to Patient

Certificate No. RTIP18/_____

Certificate

This is to certify that Dr./Mr./Ms. P. VijayaLakshmi

from College of Pharmacy, Madras Medical College, Chennai

participated in the 7th National Conference on "Recent Trends in Industrial Pharmacognosy - 2018", organised by Department of Pharmacognosy, College of Pharmacy, MTPG & RIHS, Puducherry on 16th March 2018 and presented a paper in the Oral / Poster / e-Poster session, titled .....

.....

with co-authors.....

V. Gopal

Prof.DR.V.Gopal
Registrar Academic
Convenor - RTIP'18

R

Dr.R.Murali
Dean
Chief Patron - RTIP'18

ACCREDITED BY THE TAMILNADU Dr.M.G.R MEDICAL UNIVERSITY, CHENNAI WITH 10 CREDIT POINTS.

Dedicated my
beloved my family
And Almighty god

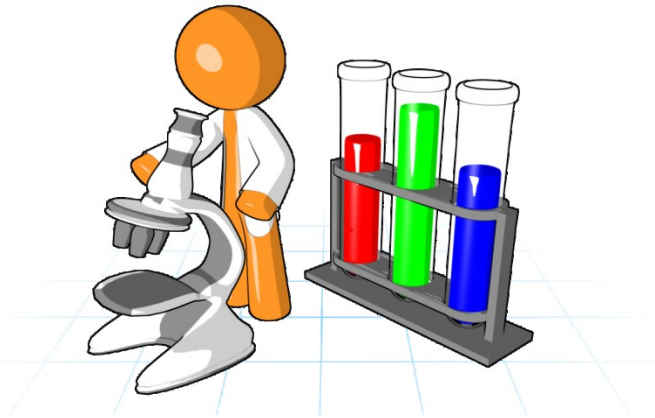
Introduction

Review
Of literature

Plant profile

Aim and objectives

Plan of work



Materials and methods

Results and discussion

Summary and conclusion

Reference

Annexure